Water quality improvement and the promotion of cultured oyster production by artificial upwelling

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ABSTRACT

Filter feeding bivalves are key organisms for the development of sustainable aquaculture, because they do not require artificial feeding and depend mostly on the primary producers in their environment. Thus, bivalve production is extremely susceptible to the characteristics of the farming location. Significant mass mortalities have been reported worldwide in a number of cultured bivalve populations. These mortalities mainly occur during summer as a consequence of deteriorating water quality conditions and stressful synergetic effects. For example, in Omura Bay, Nagasaki Prefecture, where shellfish farming is performed commercially, several episodes of red tides and hypoxic water formation have been reported in summer, as a result of poor water exchange.

Shell movement responses of oyster *Crassostrea gigas* exposed to different temperature and oxygen concentration were investigated in order to identify stressful conditions for *C. gigas*. The measuring equipment consisted of a magnet and sensor attached to each oyster shell valve, indicating shell movements by the variances in the magnetic field. Extreme temperature led oysters to close their shells, while spiking activity positively correlated with increasing temperature ($R^2 = 0.93$, $p < 0.01$). Being oyster regulators, they showed bigger valve gap at levels of dissolved oxygen saturation under 40%, in an attempt to increase filtration to obtain more oxygen. However, this response was unobserved at 5% dissolved oxygen (DO) saturation levels. Considering filtration activity is indispensable, temperature range around 15-20 ℃ and levels above 60% of dissolved oxygen saturation would be the optimum conditions for *C. gigas*. These results illustrate situations in which oysters would be vulnerable due to environmental stress, highlighting the necessity of mitigative managements.

Because the summer conditions of Omura Bay were shown to be outside the oyster optimum range of temperature and DO, an artificial upwelling system was installed as mitigative management for increasing water quality for aquaculture. The aim of this study was to investigate the efficiency of an artificial upwelling in improving water quality in a summer stratified bay, by inducing mixing as well as positively affecting biogeochemical processes such as primary production. Sampling

consisted of CTD profiles, carbon and nitrogen isotopes of particulate organic matter and temperature monitoring mooring system concentrated within 2 km from the aeration point. A sampling station in the centre of the bay was considered as control. At the aeration centre, the water column was thermally homogeneous, and had higher chlorophyll *a* and lower DO concentration compared to the sampling at bay centre. Just north of the aeration point the water column was less stratified than the other sampling locations along the mooring system, because of the wind or northward current. $\delta^{13}C$ at aeration centre was slightly higher and C:N ratios were smaller than other locations non-affected by aeration, probably due to enhanced primary production.

An artificial aeration was installed in an oyster farm to investigate the effects of artificial upwelling on oyster condition, through environmental parameters and their connection with oyster overall condition. The aeration was performed from the sea bottom in an oyster farm in Seihi area, Omura Bay, during two summer seasons in 2011 and 2012. Oceanographic parameters (temperature, salinity, dissolved oxygen concentration, chlorophyll *a* concentration, and suspended solids) and oyster performance (growth, survival, condition index, and glycogen levels) were monitored monthly at different stations at increasing distances from the aeration. The aeration was shown to be efficient in improving water conditions for oyster farming, especially in the beginning of summer, by locally decreasing temperature by approximately 1°C, redistributing nutrients, and increasing diatom biomass. The condition index of oysters was negatively related to the distance from the aeration point. Furthermore, a reproductive season, when the aeration could not overcome high temperature and reduced oxygen, resulted in poor oyster health (condition index and glycogen levels decreased in September). The results indicate that the aeration can improve bivalve cultures, if it is performed at a rate that overcomes hypoxia formation and high water temperature throughout the summer period.

Studies on oyster performance using real commercial data and their relationship with environmental parameters are important to support mitigation plans for common farming problems. To identify the important period of an artificial upwelling, oyster crops were studied together with temperature and food availability. The available production data of a Brazilian bivalve farm in four years (2005/06, 2006/07, 2007/08, 2008/09) was analyzed as a case study. Performance during initial

grow-out stages (seed to juvenile) was critical for final crop yield. Temperature was the main factor affecting survival in these initial stages with a trend of negative correlation. On the other hand, oyster development rate was significantly and positively affected by chlorophyll *a* concentration. Because decreased temperature and increased chlorophyll *a* are among the positive results of artificial upwelling systems, this study indicates that the aeration should be performed especially in the early farming stages for better oyster aquaculture production.

Temperature and dissolved oxygen levels in oyster aquaculture fields are often outside the optimum conditions for oysters in summer, thus mitigative managements are required to guarantee good water quality in local oyster farms. It was revealed that the artificial aeration can decrease water column stratification, decrease temperature, increase phytoplankton biomass and quality, and improve the oyster condition index, indicating a possible commercial applicability in the future. Additional research is necessary to explore new aeration rates and experiment designs for better oyster production, because aeration requirements can differ among target species and locations.

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Chapter 1.

General Introduction

1.1 Background

1.1.1 Shellfish aquaculture in a changing world

Aquaculture is defined as the managed growth of aquatic organisms with at least one stage in the water and is regarded as the only hope for substantial expansion of aquatic food production in the foreseeable future (Davenport et al., 2009). Nowadays humanity´s great concerns are the changes in our environment such as global warming, oceans acidification, increasing population and the uncertainties of an unknown future that comes with it. One of the main questions of a world facing changing climate is how we will be able to produce enough food to maintain a world expanding population in a sustainable manner (Takeda, 2002).

Agriculture is not a suitable approach for increasing food production since land for agriculture is scarce, therefore the attention is turned to aquatic resources mainly related to the sea. While wild fisheries have not been able to increase production in the last few decades due to over exploration of stocks, aquaculture has increased as the fastest growing food-production sector (Fig. 1-1). However, many negative points have surfaced related to aquaculture practices. The most critical point is the production of food pellets for high food-web positioned animals which use fish oil in its composition, thus artificial food production demand resources from wild stocks. On the other hand, aquaculture of filter feeding bivalves takes advantage of natural water productivity, converting marine particulate organic matter into premium protein of high nutritive value. Shellfish is regarded as an extremely good cost-benefit culture and as potential species for solving the world food problem.

Shellfish culture although relatively simple to perform, primarily require a productive environment with adequate availability of phytoplankton and particulate organic matter (POM), as well as proper range in abiotic conditions such as temperature and salinity to achieve faster growth. One of the most environmental widespread and globally cultured species is the Pacific oyster, *Crassostrea gigas.* In Japan for example, *C. gigas* accounts for almost 50% (200,298 ton) of the marine shellfish production, reaching values of USD 371.2 billion in 2010 (FAO, 2013a).

1.1.2 Summer mortalities

Oyster summer mortality is a globally widespread phenomenon that causes losses of production up to as much as 50%. The phenomenon has been first observed in the 1940s in Japan. However, still nowadays it is subject of continuous researches which try to find its possible causes. Initially, mortalities were attributed to eutrophication, mainly enriched seawater combined with high temperature. Other bivalve mortality precursors could be oxygen depletion (Akagi and Hirayama, 1991), high temperature, eutrophic conditions (Malham et al., 2009), virus infections (Burge et al., 2007), fouling organisms (Alagarswami and Chellam, 1976), and carbohydrate anabolism linked to reproduction (e.g., Cotter, et al., 2010), including even shell asymmetry (Fréchette et al., 2003). Nowadays the most accepted theory is that complex succession of events and synergetic factors would lead oysters to inevitable mortality in summer (Chávez-Villalba et al., 2007; Malham et al., 2009).

Oysters that survive summer period are often stressed and weak, which usually influences oyster quality, such as oyster size, quantity and taste of meat, etc.

Overcoming summer mortalities and increasing oyster quality is a need for achieving profitable mariculture outcomes. Therefore, there is a strong desire for new effective technologies and managements.

1.1.3 Artificial upwelling applicability

Artificial upwelling has been recently described as a method to enhance water quality and thus, increase production and quality of products in mariculture established areas (Berntsen et al., 2002). Positive effects of the induced upwelling flux would include reduced hydrographical stratification promoted by turbulence, enrichment of superficial layers due to nutrients brought up with the deep water and lower water temperatures (e.g., Strand, 1996). In recent years, experiments and models have been developed in order to improve water quality and favour phytoplankton blooms in bivalve farming locations (Willianson et al., 2009). In a Norwegian fjord, artificial aeration was designed to evaluate the growth stimulation of non-toxic algae used as food by mussels through the upwelled nutrient rich water (Handa et al., 2013). Water brought up from lower layers successfully increased the non-toxic algae biomass by the increasing of silicate input to euphotic layers. These results were considered promising for improving non-toxic algae biomass as food for cultured mussels. As a result, suggestions about the artificial upwelling applicability for aquaculture have been widely spread, although artificial upwelling-shellfish relationship has not been directly assessed by any of those works.

1.2 Study area

1.2.1 Overview

Omura Bay is an enclosed shallow estuary with a surface area of 320 km^2, length of 26 km and mean depth of 19 m in Nagasaki Prefecture (Fig. 1-2). The bay receives fresh water from rivers mainly in south-eastern bay head. Water exchange with outer ocean is limited by two narrow straits: Hario and Haiki, with approximately 200 m

and 20 m width, respectively (Akagi &Hirayama, 1991). Strong tidal currents of over 4 m s^{-1} occur according to the tidal cycle around Hario strait. However, inside the bay tidal currents are extremely weak (0.1-0.2 m s^{-1}; Takahashi et al., 2009). Accordingly, bay area can be divided in two separate regions whose physical characteristics are different. The first is the smaller region from the straits to the location of the narrowing of the bay width, with average 30 m depth, in which the stratification does not occur even in the summer and anoxic water is never observed. The second one is delimitated from the narrowing of bay width towards the inner bay, with a mean depth of 16 m. The currents are weak and consequently water is stratified in summer and oxygen-depleted water is formed in the bottom.

The bay is used commercially for both fishing and aquaculture, including shellfishes such as Pacific oyster (*Crassostrea gigas*), Pearl oyster (*Pinctada fucata*) and Noble scallop (*Mimachlamys nobilis).*

1.2.2 Hypoxic events

Even though Omura Bay is characterised by low nutrient levels and has a highly treated sewage (Yamaguchi et al., 2007) several episodes of red tide and hypoxic water upwelling (Sumishio) have been reported in the area, as a result of poor water exchange (Takahashi et al., 2009; Wada *et al.*, 2012). In general, water quality in urban areas deteriorates due to inflow pollutants from the cities. However, in Omura Bay, the average concentrations of phosphorus and total nitrogen are very small (in 1997 T-P = 0.6 µM and T-N = 19.3 µM) in comparison with other urban areas. Hypoxia occurs especially during summer when the water stratification is high, beginning in the centre of the bay. Bottom layer in the bay can reach more than 27 °C temperature, and water is many degrees warmer in the surface (Takahashi et al., 2009). The formation of thermocline prevents supply of oxygen in the bottom layer. Distributions of oxygen–deficient water vary from year to year influenced by variations in the weather (ex., wind; Takahashi et al., 2009) and oceanographic conditions (Fukumoto and Kobayashi, 2005; Fig. 1-3). The initial distribution of cold hypoxic water coincides with

the formation of a stagnant area generated by flow fields in the bay centre, and hypoxic water is later transferred towards wind direction (Takahashi et al., 2009). In these anaerobic conditions, nutrients (PO_4 and NH_4) can be released from the bottom sediments. As a result, concentrations of nutrients and oxygen were inversely correlated (Akagi and Hirayama, 1991). Previous study has suggested that aeration from the bottom or dredge of the sediment would help reducing the formation of oxygen-deficient water mass and supposedly increase the area production (Akagi and Hirayama, 1991).

1.2.3 Previous attempts to minimize summer related problems

Oyster farming in Omura Bay is believed to be severely affected by high temperature and hypoxic conditions in the summer, when more than half of the oyster production is lost due to mortalities. This leads to an impact on the earnings of many families that depend on the farming activity as an important complementary income. Oyster mortality and lower final production in the area is believed to be caused by hypoxic waters linked to high temperatures in summer. In order to reduce the mortality of cultured oysters, a pilot study has already been performed in a small bay within Omura Bay involving closed systems called ‘mesocosms’, in which water was aerated from the bottom (Yamaguchi et al., 2007). Experimental tests combining mesocosms with and without aeration and with and without oysters were conducted. Aeration kept DO in good levels even when oysters were present. The results seemed promising in relation to water condition and oyster growth in those closed aerated systems.

1.3 Purposes of this study

1.3.1 Objectives

The objective of this study was to examine the effects of an artificial upwelling on environmental characteristics and test the hypothesis that it could improve water quality in summer-stratified enclosed bays. Initially, to confirm the necessity of mitigative management in Omura Bay, laboratory experiments were performed aiming to characterize oysters shell behaviour under summer temperature and oxygen conditions and identify situations that would induce stressful behaviour. This information could allow a better understanding of pre-mortality events and provides additional knowledge to support the research and development of mitigative management in an oyster farm. Therefore the research is of extreme importance. Aeration was expected to improve environmental conditions and promote an overall better condition of oyster *C. gigas* due to aeration system. It was expected that the aeration would not only increase DO concentration but possibly decrease temperature, induce mixing redistributing nutrients from the bottom rich layers, and keep suspended particles in the water column. Thus, aeration would also improve hydrological conditions for Pacific oysters and increase their food availability (Fig. 1-4).

1.3.2 Structure of the thesis

In order to achieve the objectives, this research was developed at the following main fronts:

- Identification of main temperature and oxygen situations that could cause oysters stress and lead them to death (Chapter 2)

- Assessment of the efficiency of the artificial upwelling in minimizing stratification and improving water quality in Omura Bay (Chapter 3)
- Identification of possible improvements on oyster condition due to artificial upwelling (Chapter 4)
- Exemplification of how the environmental characteristics of cultured site affect oyster production in a study case in Brazil and how aeration could be applied (Chapter 5)

On top of these, on ‘Chapter 5’ it will be discussed how artificial aeration could be useful in other bays in the world, as an example Brazil. The general discussions, conclusions and future perspectives constitute the ‘Chapter 6’.

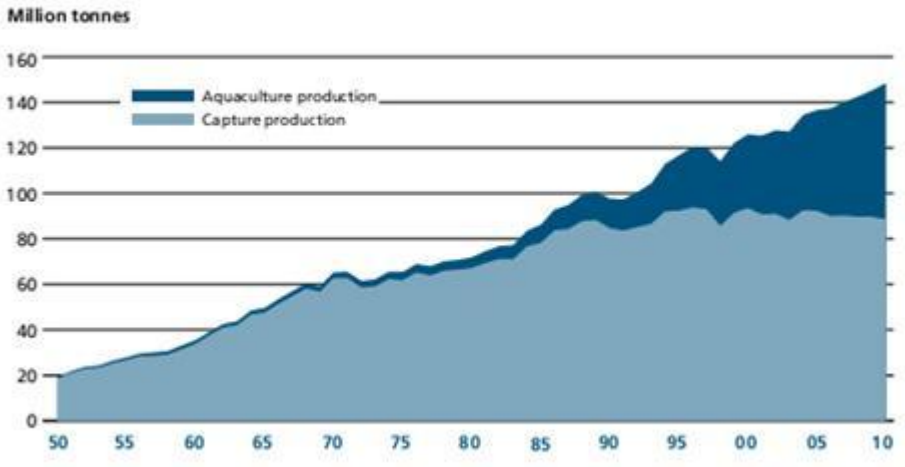


Figure 1-1: World capture fisheries and aquaculture production (SOFIA, FAO 2013b).

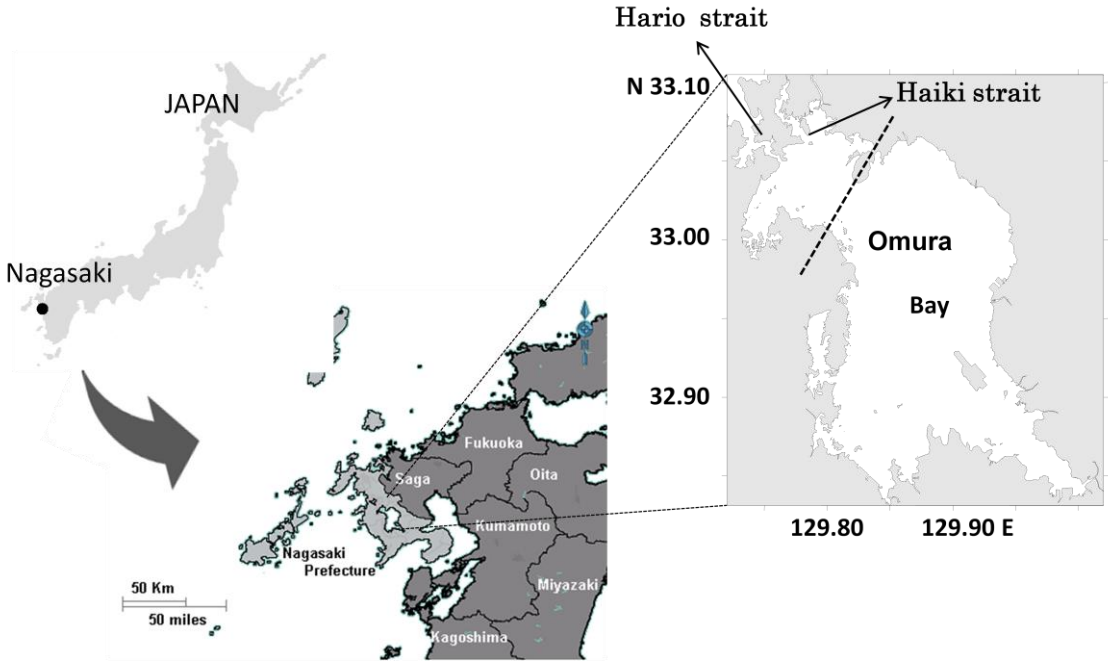


Figure 1-2: Location of Omura Bay in Nagasaki Prefecture. Dashed line separates the year-round mixed region (upper part or bay mouth) and the summer stratified part (lower part or inner bay).

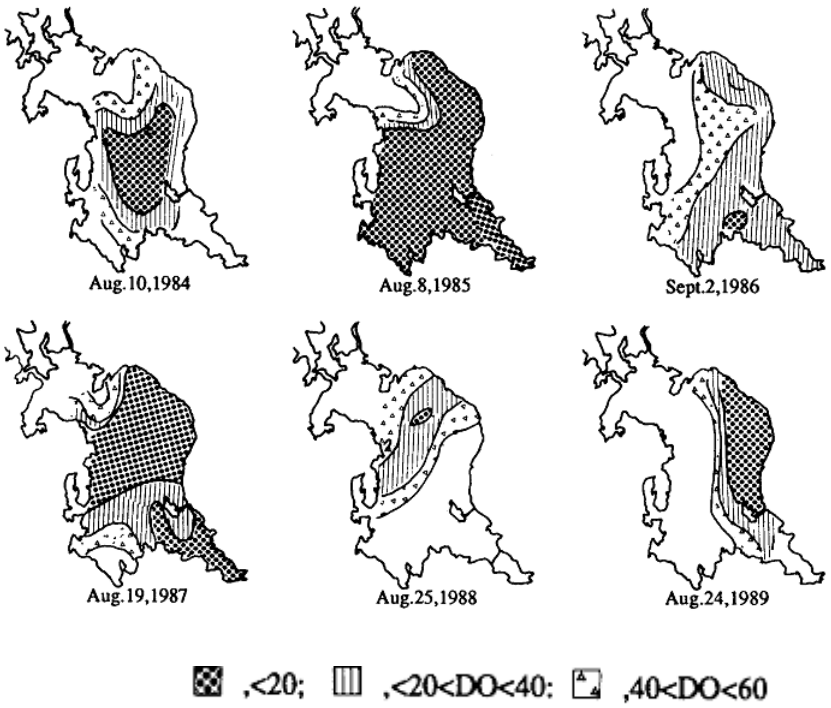


Figure 1-3: Historical horizontal distributions of oxygen-deficient bottom layer water mass in August months from 1984-1989 (Akagi and Hirayama, 1991). Legend represents different levels of DO (% saturation).

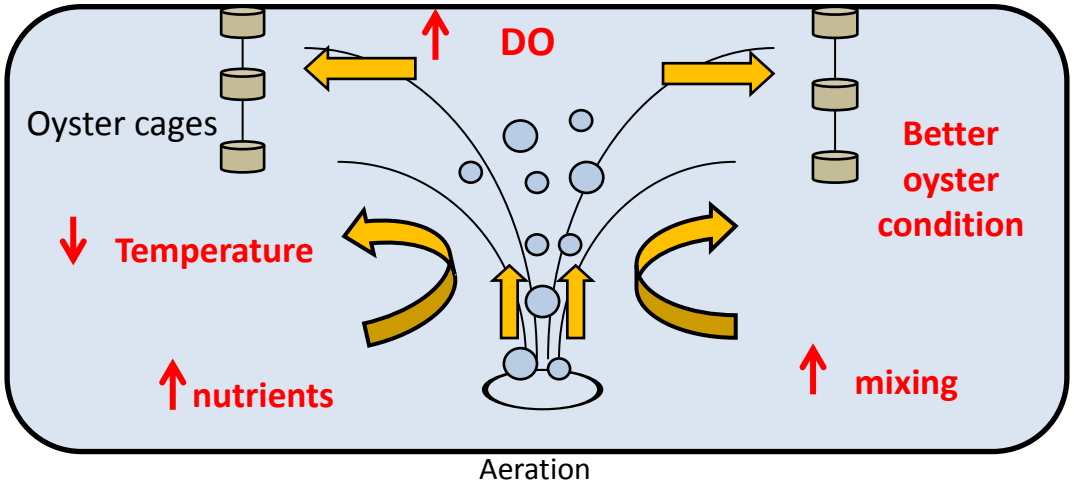


Figure 1-4: Expected aeration effects.

Chapter 2.

Shell movement responses of oyster *Crassostrea gigas* at different temperature and oxygen conditions

2.1 Introduction

The current understanding of the globally common problem of oyster summer mortality is based on correlations between environmental data and post mortem analyses of body chemical contents (David et al., 2005; Allen and Burnett, 2008). Many causes have been described as bivalve mortality precursors, such as oxygen depletion (Akagi and Hirayama, 1991), high temperature, eutrophic conditions (Malham et al., 2009), virus infections (Burge et al., 2007), fouling organisms (Alagarswami and Chellam, 1976), and combination of these conditions. Overall, it is a general consensus that oyster tend to be weak and prone to die in summer when metabolic rate and energetic demand (e.g. reproduction) are high. Extremely poor environmental conditions induce oyster stress especially during summer months.

The stressed and weakened oyster survivors are more susceptible to pathogens. Because most of shellfish pathogens are also pathogenic to humans, guaranteeing good farming conditions for oysters intended for human consumption implies not only in their health but also our own (Lacoste et al., 2001). In spite of that, only a few studies have been conducted on the immediate response of oysters to different stress-inducing summer environmental situations while they are still alive (e.g., Tran, 2010).

Shell movements of bivalves are a good indicator of immediate response, and have been studied in connection to possible changes and stressful characteristics of the habitat, such as detection of toxic algal blooms (Tran et al., 2010), filtration rates (Riisgard et al., 2003) and circatidal rhythm (Mat et al., 2013). The opening and closing of valves are defensive reaction to external stimuli as well as a response to deteriorating environmental conditions (Nagai et al., 2006). Thus, shell movements can promptly indicate variations in the health of both the bivalves and their environment (Schmitt and

Rosa, 2011).

In a study of Pacific oyster *Crassostrea gigas* exposure to both non-toxic and toxic algae (*Alexandrium minutum*), Tran et al. (2010) found out oysters had a normal shell movement behaviour when fed non-toxic algae, but abnormal in a toxic algae condition. Eventual closures of the shell in apparent no stressful conditions are related to pseudofaeces expulsion. However, an intense shell activity is regarded as an abnormal pattern. While high frequency of gaping expressed by opening and closing of valves (spikes), indicates an uncomfortable situation for the bivalve, sustained closure usually occurs in adverse conditions or events such as extremes of temperature or salinity, attack of enemies, air exposure and others (Yonge, 1960). Furthermore, fully opened states and tightly closed valve states have been proved to control maximum filtration rates and represent inactive filtratings, respectively. Therefore, the valve gap is positively related to filtration rate (Tran et al., 2000, Riisgard et al., 2003), and thus indirectly related to oxygen and food uptake.

The farming of the commercially important Pacific oyster is one of the fishery resources in Omura Bay, Nagasaki Prefecture, Japan. In summer nonetheless, losses of oyster production caused alarm in the oyster industry. Oyster mortality has been reported for several enclosed bays around the world. In Omura Bay they are mainly attributed to hypoxic conditions and high temperature (Soletchnik et al, 2005; Malham et al., 2009).

Oysters have evolved metabolic adaptation to life in intertidal zones, which are acknowledged as highly changeable environments. For instance, the closure of valves isolates the tissues from the stressful external environments and subsequently decreases metabolic rates, increasing the accumulation of metabolic bio-products and acidosis as well (Lombard et al., 2013). Although oysters can survive in a wide range of temperature, they are negatively affected by summer high water temperature (Gagnaire et al., 2006). Most coastal populations can tolerate exposures to low DO concentrations. However, prolonged exposures to speculated levels of less than 60% oxygen saturation may result in altered behaviour, reduced growth, adverse reproductive effect and mortality (Karim et al., 2002).

Shell movement reactions to different summer adverse conditions can provide clues on which specific factors intensely disturb the bivalves leading oysters to death. Nonetheless, shell behaviour responses to summer high temperature and low dissolved

oxygen concentrations remain relatively unknown.

2.2 Objectives

Omura Bay summer mortality case was considered as a motivation for the development of this work. The purpose of this research was to identify changes in shell behaviour of *C. gigas* at different temperature and dissolved oxygen concentrations, which could indicate different levels of stress. Such information is important in order to increase our understanding of pre-mortality scenarios. Furthermore, it may assist in determining main situations that could contribute to oyster mortality, and provide knowledge for the development of mitigative actions.

2.3 Materials and methods

2.3.1 Experimental setup

C. gigas were collected in an oyster bed in Maizuru, Kyoto Prefecture, coastal side in the Sea of Japan. Oysters were transferred to tanks in a laboratory filled with artificial sea water (Marine Merit, Osaka, Japan, salinity = 30) and left acclimating for at least 48 h before the experiments. The size of oysters was between 6 cm and 10 cm shell height, which matches the cultured oyster size in summer in Japan.

Eight random oysters were chosen for each experiment. Each animal was coupled with a set of calibrated sensor and magnet attached to each of the valves and connected to a valve gap measurement logger (SL-100 series, Tokyo Sokki Kenkyujo Co., Ltd.). Calibration was performed to each bivalve individually, in a way that the 10 mm between sensors attached in a fully closed oyster would equal to the initial zero value of the valve gap (Fig. 2-1). Magnetic field variations between the sensor and magnet would reflect opening and closing movements of oyster valves as described in

Nagai et al. (2006). Oysters were fed a commercial algae solution of *Chaetoceros gracilis* (Beckman Coulter) daily, excluding 12 hours previous to and throughout the experiments. This was done to prevent effects of instantaneous opening of the valves during and after the feeding and minimize interference of valve movements due to pseudofaeces expulsions (Yonge, 1960; Riisgard et al., 2003). Oysters rested for at least 12 h in the initial conditions before the experiment started. The experimental room was kept at dim light and out of any human disturbance in the surroundings of the experimental tank. The tank was aerated in a way that the bubbles did not come into direct contact with the oysters.

Temperature was controlled with a set of a heater (Nisso Power Safe Pro 100) and a cooler which also functions as a thermostat (Rei-Sea Cooler RZ 110Y). Tanks were equipped with aquarium pumps for oxygen (O_2) and nitrogen gas (N_2) to promote different oxygen levels (Fig. 2-2). Temperature and dissolved oxygen (DO) concentrations were measured with YSI Professional Optical Dissolved Oxygen instrument.

In each experiment, temperature was gradually raised between the set temperatures of 10 °C, 15 °C, 20 °C, 25 °C and 30 °C (Fig. 2-3), while DO was kept constant at one of the six saturation levels studied (100%, 80%, 60%, 40%, 20% and 0%). Although low temperatures are not consistent with the summer condition, they were also tested aiming to examine the shell behaviour under various conditions of temperature versus DO. The oyster valve response was continually recorded, even between main conditions, however the valve measurements (M) for the main conditions were considered only after one hour acclimation (A). After the experimental period the temperature was set back to 20 °C (a believed *optimum* temperature) in order to check for recovery to the normal condition (Fig. 2-3).

Temperature and dissolved oxygen concentration and respective standard deviations (s.d.) during the experiments are shown in Table 2-1. The experiment conditions were well controlled, although increases of temperature usually cause variations in DO (mg L^{-1}).

Oyster responses were analyzed by three parameters: valve gap (amplitude of shell opening), number of spikes (rapid closing/ opening movements (G_f) returning to previous valve position (G_0); $\Delta G > 0.5$ mm), and minimum valve gaps. Micro-activity

(opening and closing movements with amplitude < 0.5 mm), closures and closed states (valve gap close to zero but not equal to zero) were also identified. Examples of parameters identified during the experiments are shown in Figs. 2-4 and 2-5. All the results were expressed as means of the all oysters in each condition. For example, if a determined temperature was studied, all oxygen condition results were combined and included in that temperature. Similarly, if a determined oxygen saturation was studied, all temperature conditions were combined for that target situation.

2.3.2 Statistics

Variances of homogeneity between mean DO concentrations or temperature and oyster shell responses were verified by Kruskal-Wallis One-Way ANOVA with Post-hoc pair wise comparisons using PASW Statistics software. The significance limit was set to 0.05.

2.4 Results

The mean valve gap varied with different temperature and oxygen concentrations. The valve gaps were not significantly different among different DO levels (Kruskal-Wallis, $p = 0.28$), with similar values until 40% DO concentration. However, mean valve gap increased with the decrease in oxygen concentration in the water after a 40% saturation value was reached (Fig. 2-6a). Mean values were calculated after 10 °C data were excluded because data was considered to be significantly different from others. Temperature correlation to oyster mean valve gap was best explained by the quadratic fit ($R^2 = 0.90$, $p = 0.104$; Fig. 2-6b). After approximately 1 h exposure to temperatures of 30 °C (Fig. 2-5), oysters suddenly closed their valves. The mean valve gap was significantly lower at 10 °C than at the other temperature (Kruskal-Wallis, $p < 0.05$; Fig. 2-6b). Changes in temperature also induced valve opening response, especially in the experiment step where temperature was

increased from 10 °C to 15 °C. Responses were especially different at 40% and 20% of DO, when considering mean valve gap at increasing temperature. Mean valve gaps at those DO level seems to exponentially increase. On the contrary, at the other saturation levels, mean valve gap decreased after a critical temperature (from 25 °C, Fig. 2-7).

The number of spikes significantly increased with temperature ($R^2 = 0.93$, $p < 0.01$; Fig. 2-8a), but did not have a relationship with oxygen concentration (not shown). Frequency of spikes, however, increased at low DO concentrations, especially at 20% and 5% of DO saturation. Considering all the steps of the experiment, it was possible to identify a tendency of increased number of spikes during the acclimation stages which followed the changes in temperature (Fig. 2-8b). Spikes changed from small (amplitude = ± 1 mm) and slow (gradually returned to previous shell opening state) at low temperature, to long (amplitude = ± 4 mm) and quick ones at higher temperature. Micro-activities were also observed in the recordings but with no apparent pattern (Fig. 2-4c).

Average minimum valve gap was negative and significantly different at low temperature (Kruskal-Wallis, $p < 0.01$): at 10 °C at all oxygen levels (average = -0.31 mm). Valve gap values were also negative at 10-15 °C periods at 20% and 5% DO saturation but no significant differences were found between the minimum shell gap values and DO saturation (Kruskal-Wallis, $p = 0.57$).

In the recovery period, after temperature was set back to 20 °C for more than 1 hour, almost all oysters closed or maintained closure at all DO conditions. No oysters died during the experiment, even in the extreme conditions (Fig. 2-5).

2.5 Discussion

Summer periods are usually severe to oyster aquaculture that is performed in enclosed bays, because small water exchange results in high temperature and low oxygen concentration in the water. In order to assess the way oysters respond to different temperature and DO conditions, in vitro experiments were performed to record

shell valve movements in environments with 6 different oxygen concentrations and 5 different temperatures (Table 2-1).

The valve closing/opening is a control mechanism of filtration rates in response to different stimuli. At low phytoplankton concentrations, for example, bivalves close valves to reduce water transport through the gills and mantle cavity, thus reducing oxygen uptake and metabolism in a period of starvation (Riisgard et al., 2003). Likewise, it was found at the present work that oysters seem to try to compensate enough oxygen in the low oxygen concentration when the oxygen concentration decreases to hypoxic levels (especially at 40% or 3 mg L^{-1} of DO concentration) by filtering larger quantities of water, because average valve gap increased (Fig. 2-6a). The results observed in the present work are in agreement with the observations of Tran et al. (2000) who found the level of ventilation activity in Asian clams (*Corbicula fluminea*) is inversely related to DO level. In those clams, ventilatory flow rate can be based in the change duration of opening/closed valves, which also seem to apply for oysters. Increases in ventilation rates with decreasing DO concentrations achieves a bigger importance considering the fact that higher oxygen uptake is another response of bivalves submitted to high temperatures (Dumphy et al., 2006). These oxygen and temperature effects on valve gap could be clearly displayed in our results (Fig. 2-7).

Valve gap, however, slightly reduced at high temperatures with the minimum DO concentration, below 5% saturation (Fig. 2-6a). This is a clearly different response from that obtained at 40% and 20% DO at increased temperature (Fig. 2-7). The tolerance to hypoxic conditions in oxyregulator bivalves, such as *C. gigas*, is based on a mechanism of ventilation. However, this mechanism remains functional until a threshold level, below which bivalves cannot maintain their oxygen consumption rate and their metabolism is reduced. For *C. gigas* this threshold was reported to be from 2 to 3 mg L^{-1} (Diaz and Rosenberg, 1995; Le Moullac et al., 2007). The maximum valve gap, thus filtration rate, was observed at 20% saturation in this study. *C. gigas* slightly closed their valves at 5% of DO. Thus, in this work, the critical oxygen point, at which oysters cannot maintain their rate of oxygen uptake, was between 1.1 and 0.5 mg L^{-1}, a value considerably lower than previous studies. This would imply oysters have a higher resistance to hypoxia, and might explain why oysters did not die during the experiment.

Changes in the characteristics of environments seem to trigger shell opening on oysters. It is well reported that oysters immediately open their valves after a change in

the environment occurs, such as increase in phytoplankton concentrations (e.g., Yonge, 1960), new water exchanges in vitro experiments (Pora, et al., 1969) etc. In the present experiment, changes in temperature were followed by subtle increases in the valve gap. The valve gap gradually increased when temperature was raised from 10 to 15 °C, showing a significant switch from a previous valve-closed state (Figs. 2-4a, 2-6b).

Oysters were completely closed their valves when water temperature was as cold as 11 °C. When oysters are closed for any reason, metabolites such as acidic end products, can accumulate within tissues (Lombard et al., 2013). Moreover, in the closed state oysters deplete the oxygen stores and create a hypoxic state in the paleal cavity. The need to saturate their bodies with oxygen may have motivated the quick opening behaviour when ambient temperature was more favourable. In addition, this behaviour allowed the prompt release of acidic products to the environment. The filtration rates increased with temperature until the maximum at around 20 °C, after which filtration rate slowly started to decrease (following a quadratic fit, Fig. 6b). This means temperature can both stimulate and inhibit filtration, depending on its values. The filtration rates seem to be compromised at extremes of temperature.

It is well acknowledged that the notion of a stressful situation is indicated by the spiking in bivalves (Tyurin, 1990). The technique has been used by aquaculture companies to forecast the beginning of red tides (e.g., K. Mikimoto & Co., Ltd.). However, frequent shell movements can also indicate natural body processes and are related to different muscles from the adductor muscle. The adductor muscle located in the middle of the shell is the major area of attachment of body and valve. It consists of two muscles: the ‘quick muscle’ and ‘catch muscle’. The former contracts very suddenly and is not able to sustain contraction for long, while the latter is relatively slow but maintain position for a long time with less energy expenditure. Periodically closures of the shells occur in habitats with high concentration of suspended particles to reject the particles accumulated below the internal opening of inhalant siphon. For example, the oyster forces the rejected material (pseudofaeces) out of the inhalant siphon due to a sudden movement of the ‘quick muscle’ and by closure of its exhalant siphon (Barrington, 1967; Yonge, 1960).

During the present study recordings, it was possible to observe several sudden closures of valves were observed after returning to previous opening state or reopening. Spikes increased in number especially with increases in temperature (Fig. 2-8a). Spikes

were generally longer (large amplitude) and quicker (returned fast to the previous shell state), in higher temperatures. Spikes would not be due to pseudofaeces expulsion because oysters were not fed during the experiment. It indicates that oyster tissues are sensitive to temperature, as already suggested by Yonge (1960). Oysters might try to compensate between the closure due to sensitivity of tissues to temperature and the need of oxygen uptake, and that induces spiking activity.

In contrast with the spiking behaviour, the adductor supported a sustained closure or even sustained small gap opening with no further shell movements at extreme temperatures or low DO situations (10 °C, transition periods between 10 °C to 15 °C and in 30 °C; Fig. 2-4a). These features also appeared in the data after intense spiking activity. Especially at 30 °C it was noticeable that intense spiking was followed by a sudden closure of valves, signalling the spiking activity led to a state of exhaustion and movement stoppage. Hence, frequent spiking conditions and total closure, previously believed to show stressful situations for oysters, are by no means the only indicators of a stressful situation. This fact is especially important for elaborating an appropriate data collection protocol and analysis. Data collected only after oyster acclimation periods (e.g.: Fig. 2-3, M periods after A periods) may result in no apparent stress and no valve activity, leading to interpretation errors.

Low temperature induced sustained closures and negative gap values, suggesting an extreme effort of the muscle in pulling valves closed. Bellow the periostracum there is an outer layer (prismatic layer) formed by calcite and located only in the right valve. This layer is not entirely rigid so that, the valves close perfectly when they are drawn together by adductor (Fig. 2-9). The higher the pulling strength of the adductor is, the tighter the valves close. This explains why it is possible to obtain gap recordings showing negative closures, even though calibration was performed with oysters fully closed. Recently, the strength of shell closure has even been reported as a health indicator for oysters (Aoki et al, 2010). Negative values due to tight shell closures have also been identified to pearl oyster (*Pinctada fucata*) in a previous study that use the similar apparel (Nagai, 2006). It shows the feature is not specific for that specie of oyster, as previously suggested.

C. gigas is sensitive enough to quickly respond to changes in its environment. In the one-hour acclimation step following each temperature change, oysters spiked more than in the one-hour measuring stage which followed the acclimation. Thus, it is

believed oysters can quickly acclimate, at least partially (Fig. 2-8b). However, oysters apparently could not recover after the exposition to high temperatures, because they closed their valves during recovery period (temperature = 20 °C). Yonge (1960) discussed that oyster heart beat and tissues are permanently damaged by elevated temperatures. During the experiments, oysters were exposed to 30 °C before temperature was returned to 20 °C. This could have been the reason for the poor oyster condition expressed by closure of valves in the recovery period. Longer recordings of recovery periods should be performed to test in future studies to ensure the hypothesis.

Even though temperature seems to be a more important factor controlling oyster shell movements than oxygen concentration, synergetic effects played a whole in extreme situations. No oysters died although they experienced extreme temperature and DO condition during our experiments. Conclusively, mortalities may be related to the length of exposure assuming temperatures and oxygen concentration alone could be death precursors.

Normal oyster behaviour based on nowadays knowledge and favourable behaviour under a commercial point of view would be: reasonable valve gap opening allowing both food and oxygen uptake, minimum spiking activity and normal metabolic rate. Therefore, according to the results of this work, it is possible to identify temperature and DO situations which seem to have induced stress related behaviour: (1) 10 °C, 25°C, 30 °C and 5% DO: due to closures, negative gaps (and effort of adductor muscle), and severe spiking; (2) 40% and 20% DO levels: due to increased valve opening, probably switch to aerobioses and therefore, reduction in metabolism rate. Considering the above discussion and excluding the adverse situations previously discussed, the most suitable conditions for *C. gigas* are between 15 °C to around 20 °C of temperature and above 60% of DO concentration (Fig. 2-10). This is confirmed by the DO saturation levels (Karim et al., 2002), and optimum temperature range of 18–23°C (Le Gall and Raillard, 1988; Allen and Burnett, 2008; Bourlés et al., 2009).

2.6 Conclusions

Oyster shell movements proved to be a reliable tool to differentiate levels of stress related to temperature and DO concentrations in their environment and an optimum range for oyster normal behaviour was around 15 °C to 20 °C temperature and over 60% DO saturation levels. It is likely that oysters experience several stressful events throughout the culture period at Omura Bay, and at most farming bays worldwide, because temperature and DO ranges are often out of the optimum range found in this study. While the experiments consisted of 13 hours at different conditions of hypoxia and temperature, bivalves in farming areas experience some of those environmental conditions for several days followed during summer season. Therefore the negative effects acknowledged here might be significantly higher in situ, highlighting the urgency of studies on mitigative management techniques that could overcome the naturally adverse situations and guarantee a high crop production.

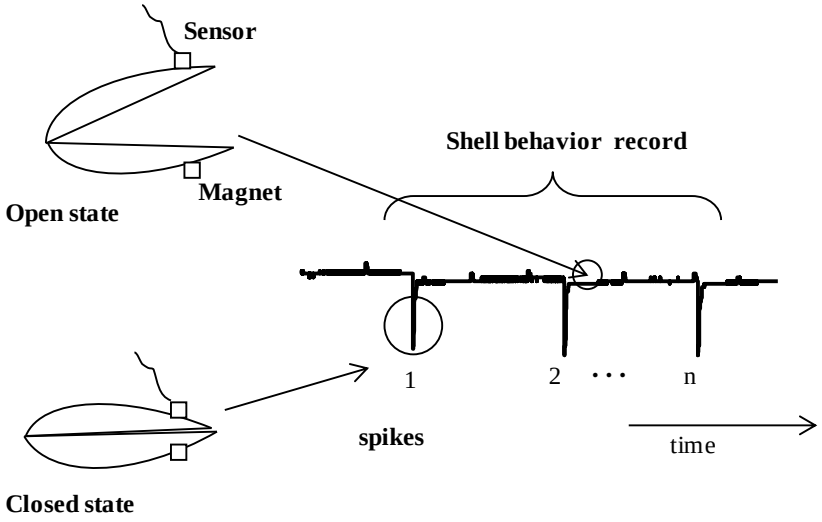


Figure 2-1: Representation of shell behaviour and related data record.

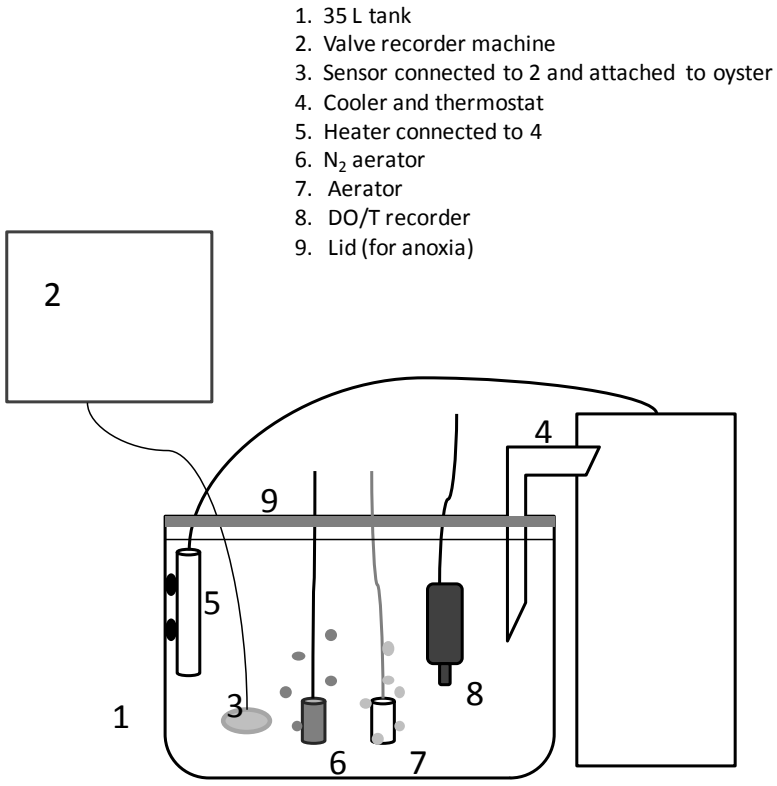


Figure 2-2: Experimental setup.

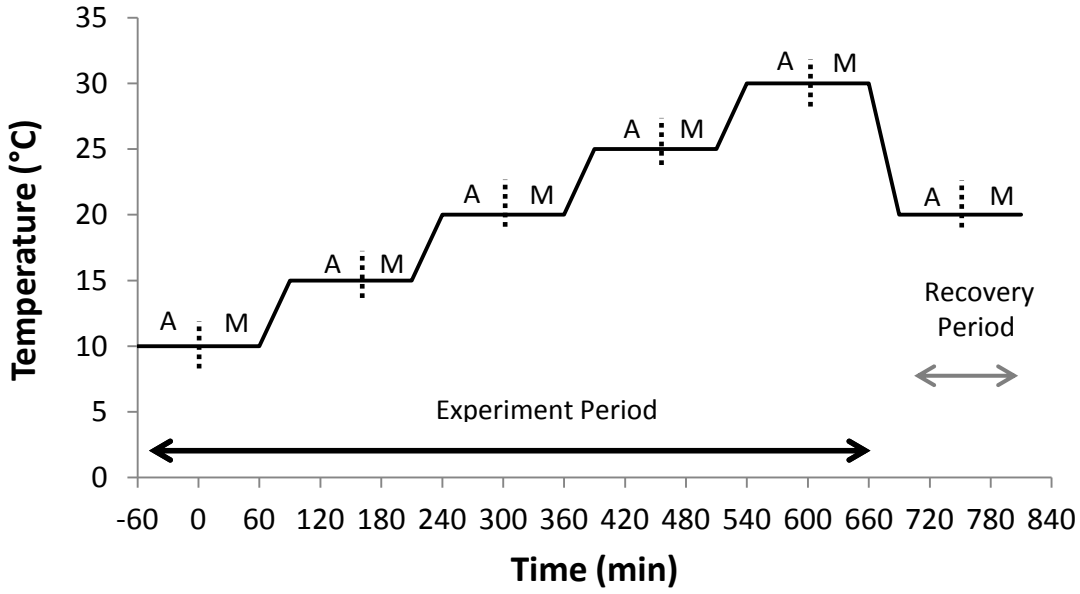


Figure 2-3: Experiment design, experimental period: showing the one-hour-long acclimation (A) and measurement (M) recordings and the recovery period.

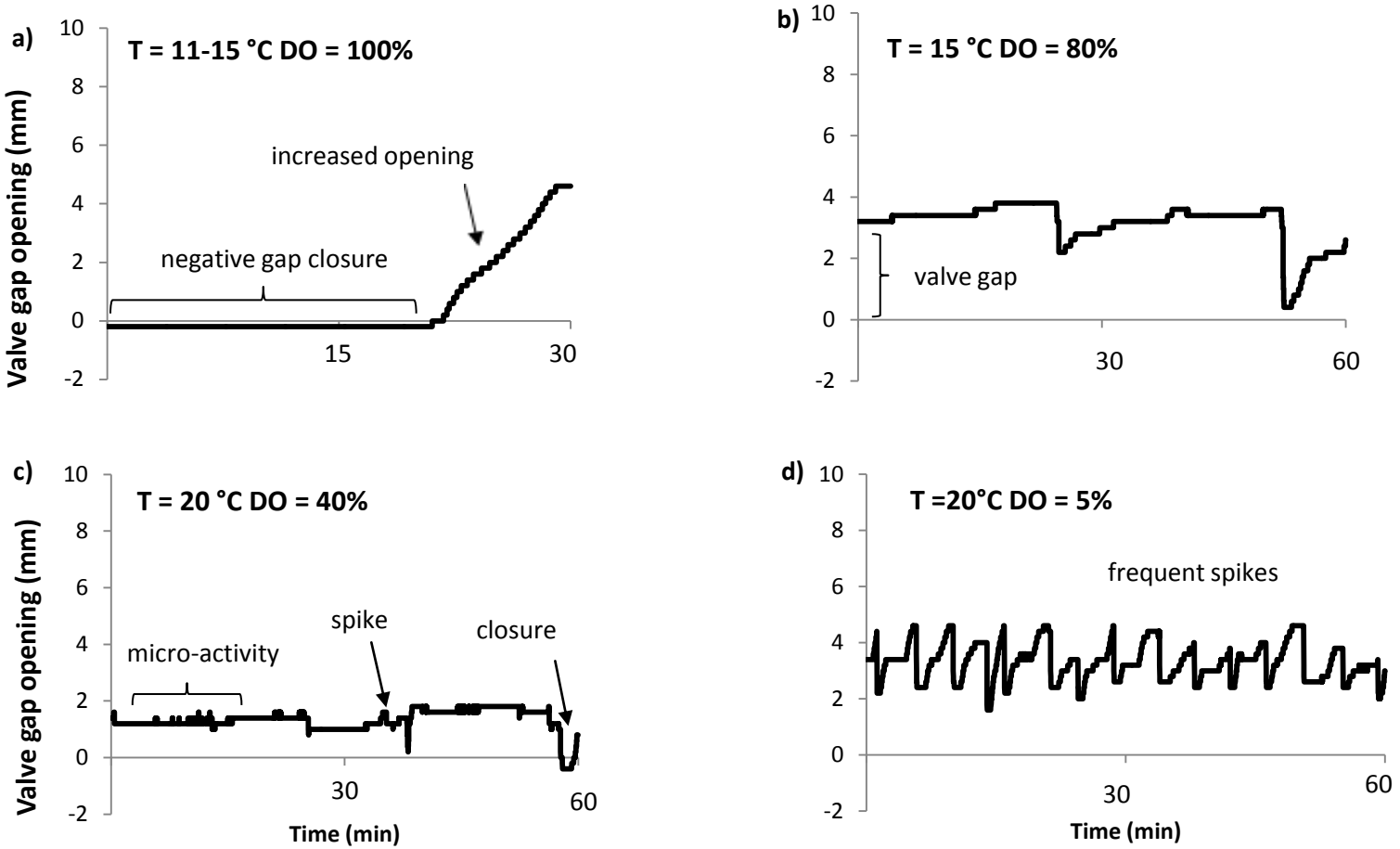


Figure 2-4: Examples of oyster shell valve activity observed at different temperatures (T) and DO conditions. **a)** increased opening and negative gap closure; **b)** valve gap (gap amplitude); **c)** micro-activity, short-quick spike, closure; **d)** frequent spikes.

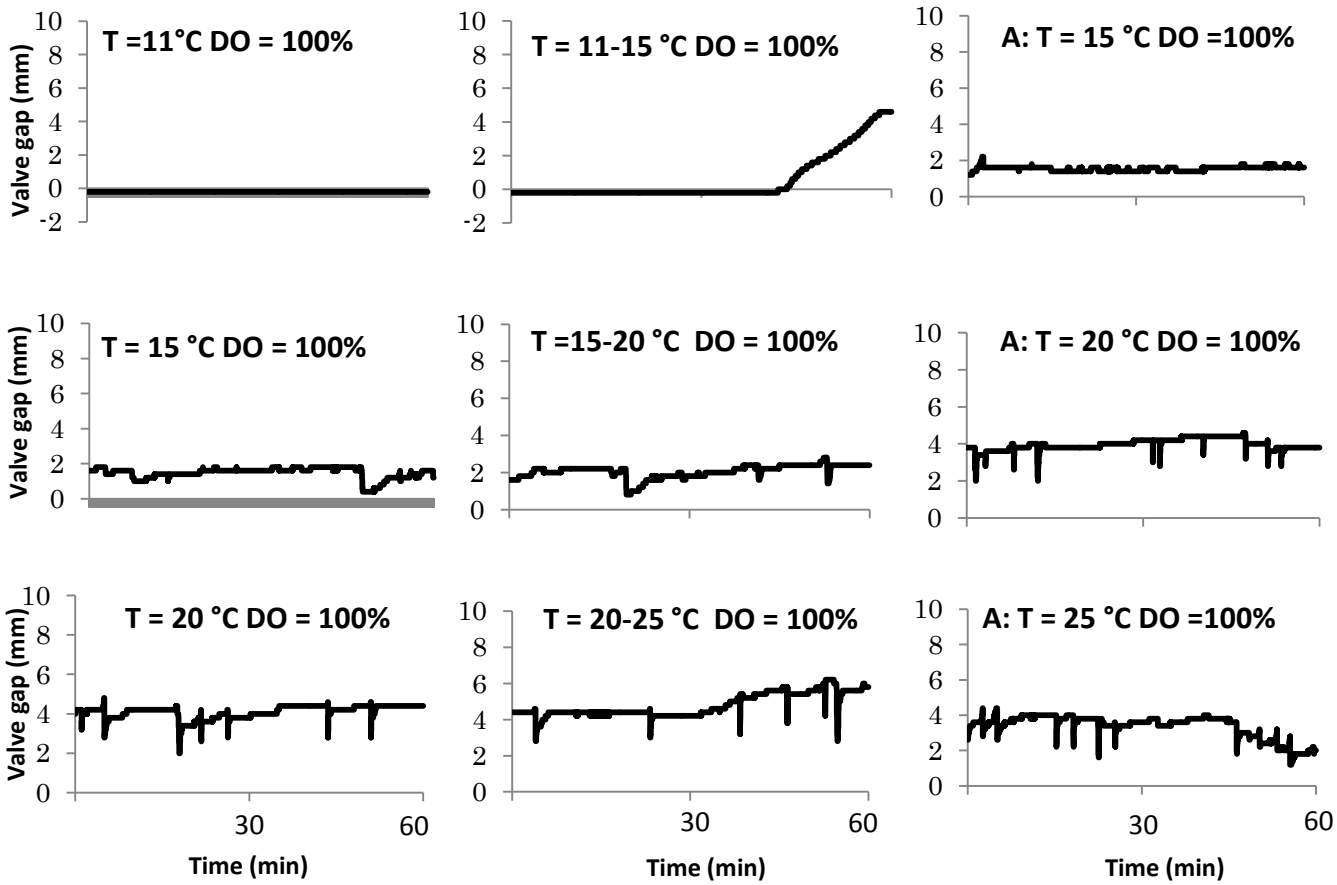


Figure 2-5: Shell behavior at different temperatures for DO saturation = 100%, example for one selected oyster in each case (not necessarily the same oyster). Acclimation periods are marked with "A".

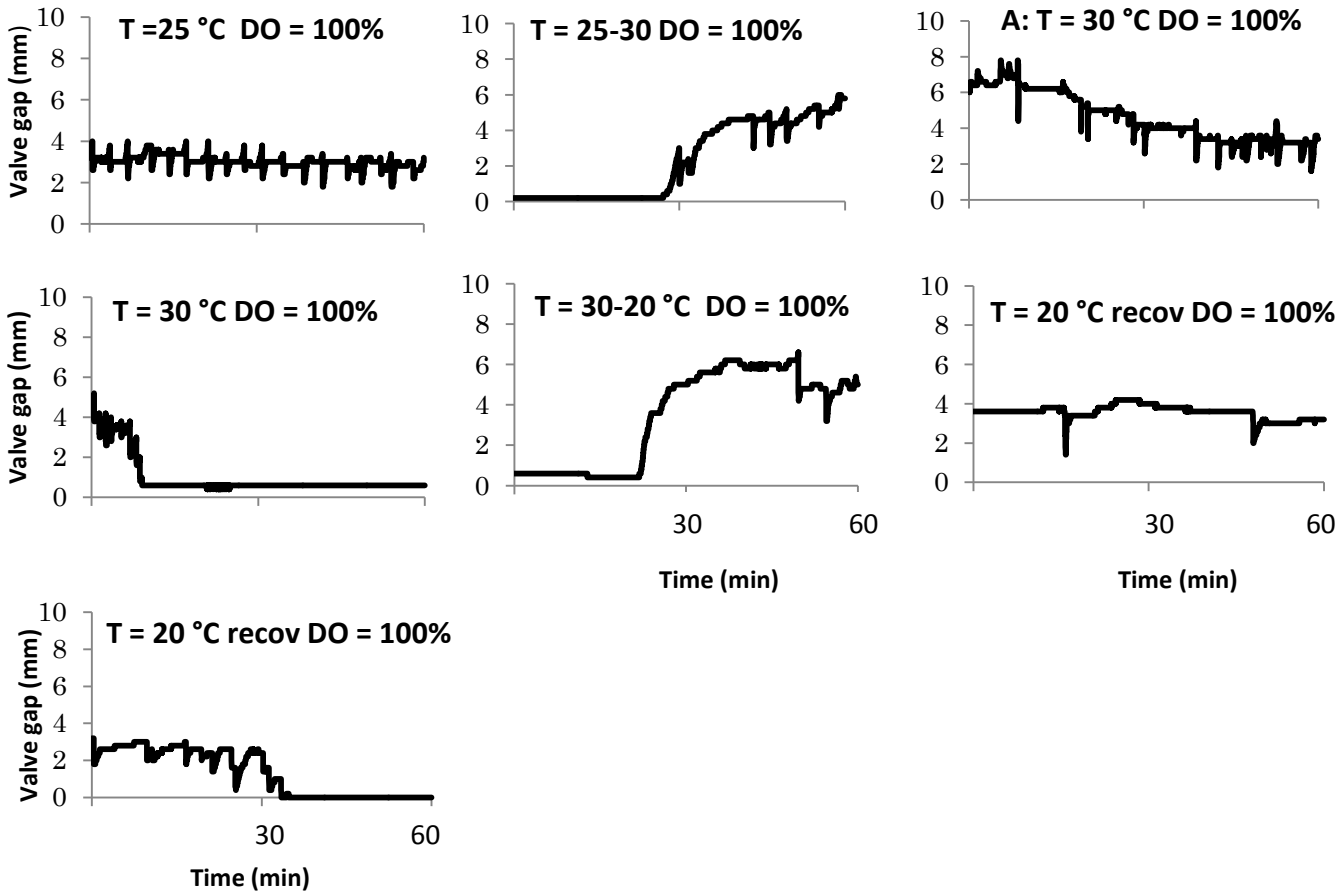
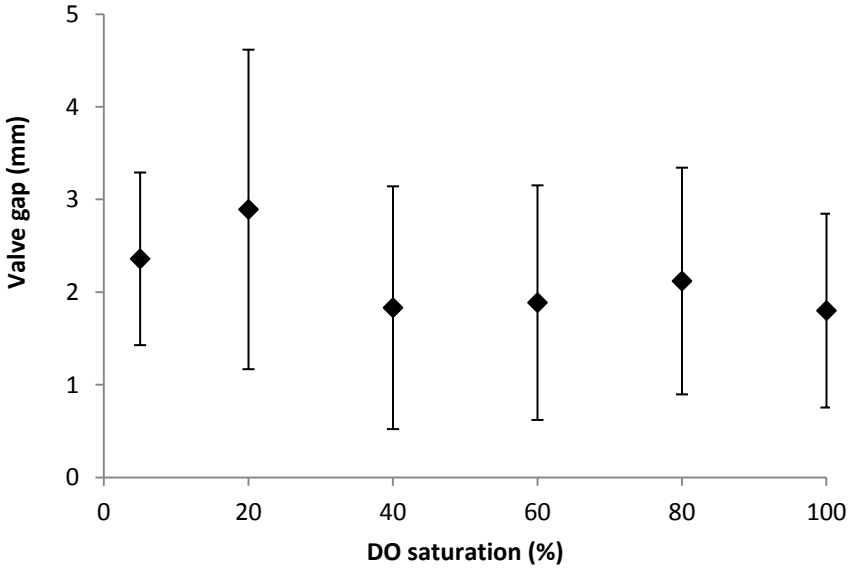


Figure 2-5 continued: Shell behavior at different temperatures for DO saturation = 100%, example for one selected oyster. Acclimation periods are marked with "A".



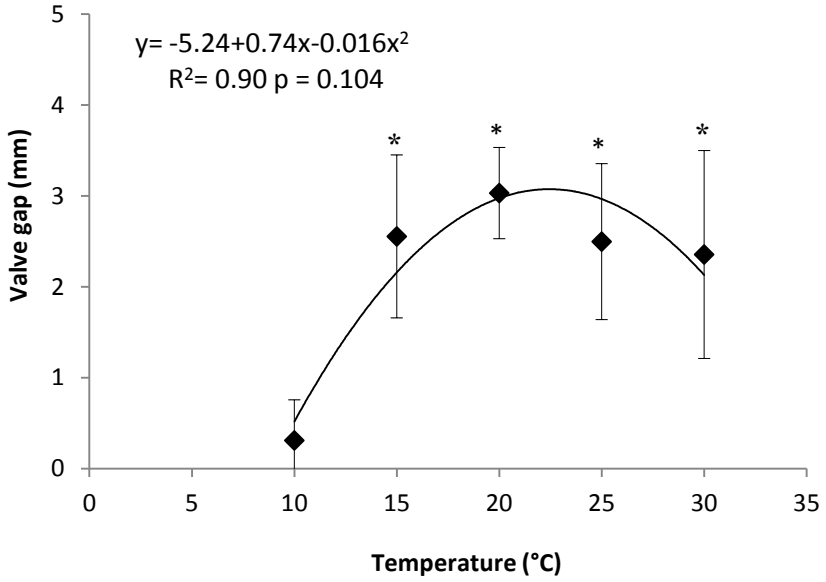


Figure 2-6: Relationships between Pacific oyster mean valve gap (mm) and **a)** dissolved oxygen concentration (%) and **b)** temperature. "*" identifies the values which are significant different than 10°C ($p < 0.05$). Vertical lines indicate standard deviations.

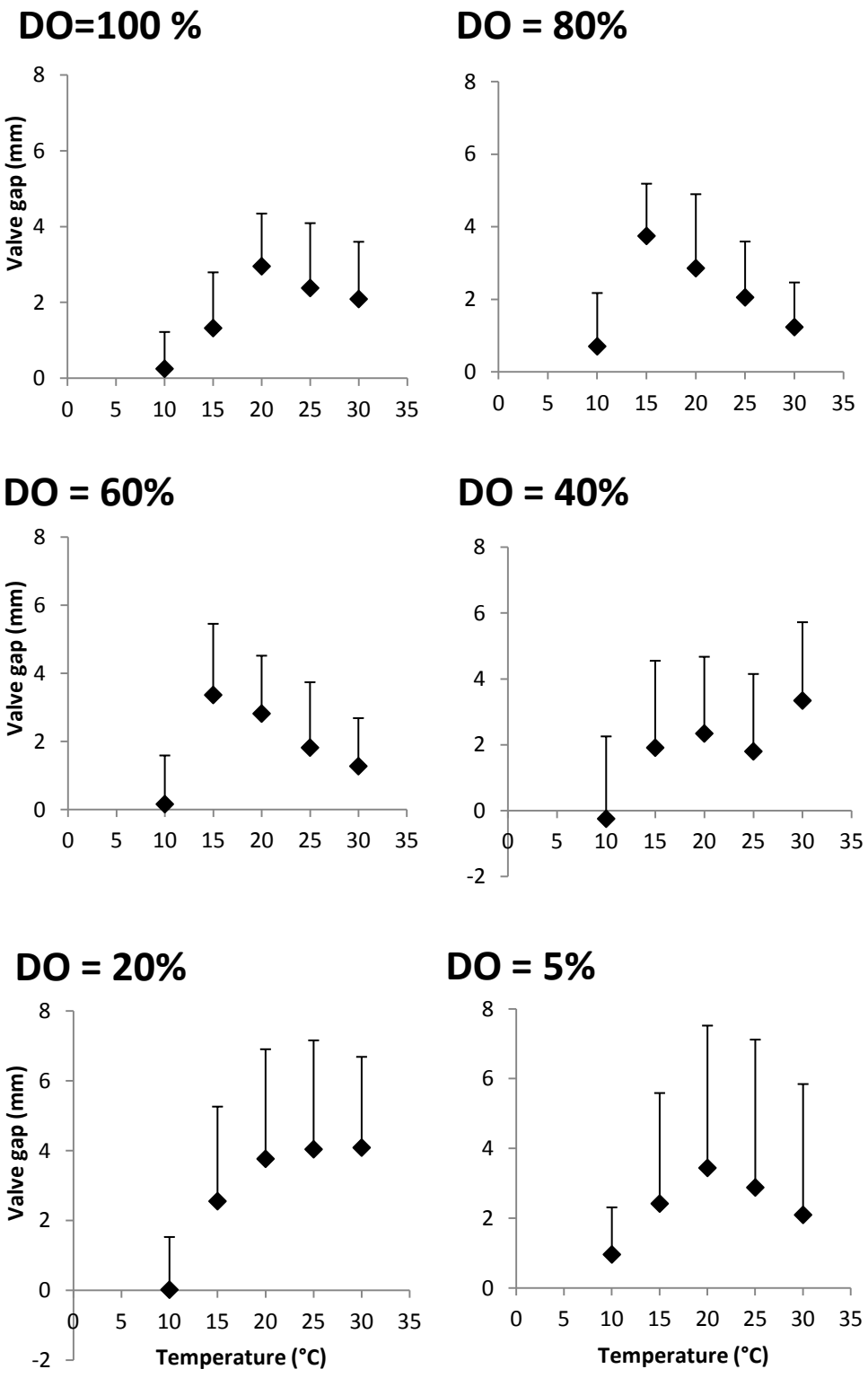


Figure 2-7: Oyster mean shell valve gap at different DO saturation levels (%) and temperatures (°C). Vertical lines indicate standard deviations.

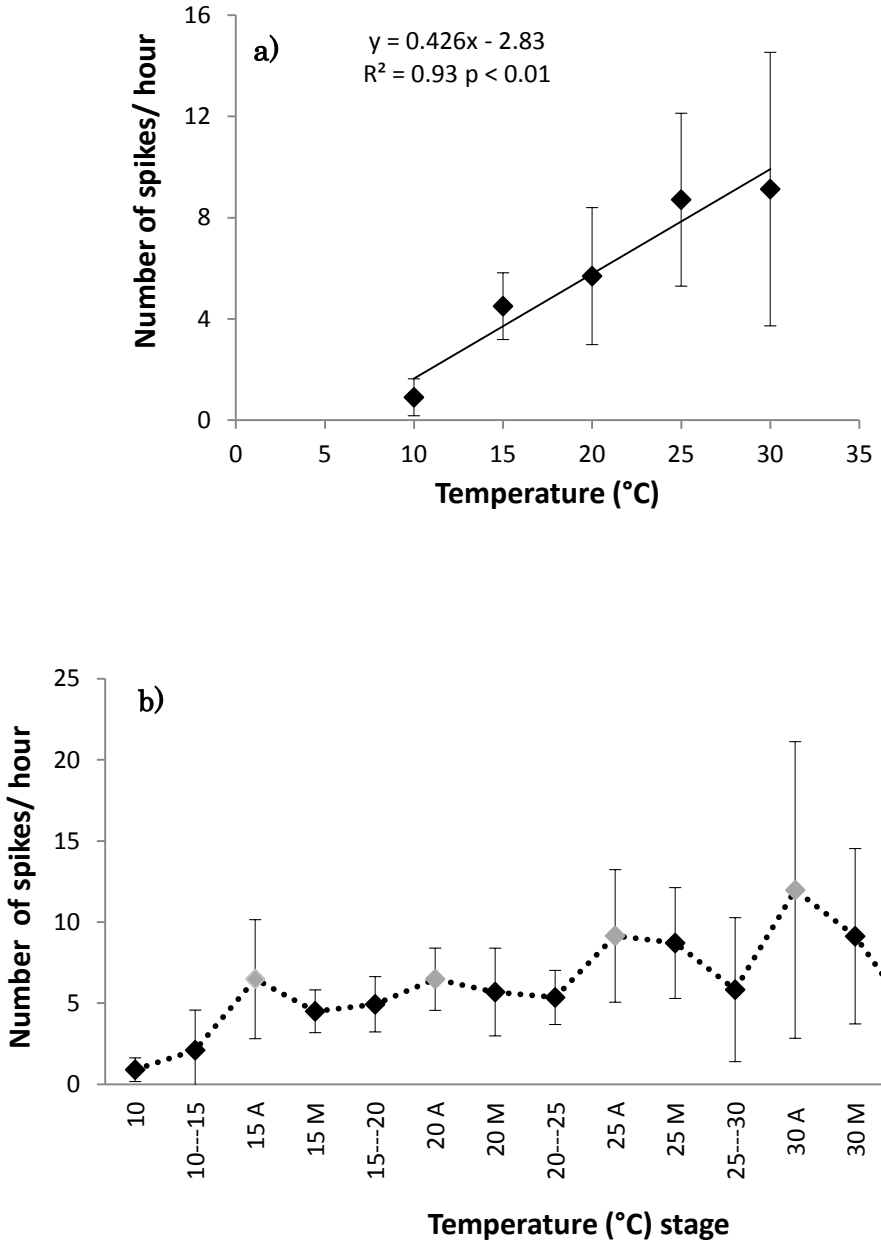


Figure 2-8: Relationships between average number of spikes and temperature (°C): **a)** in the set temperatures and **b)** in all experiment phases. Vertical lines indicate standard deviations.

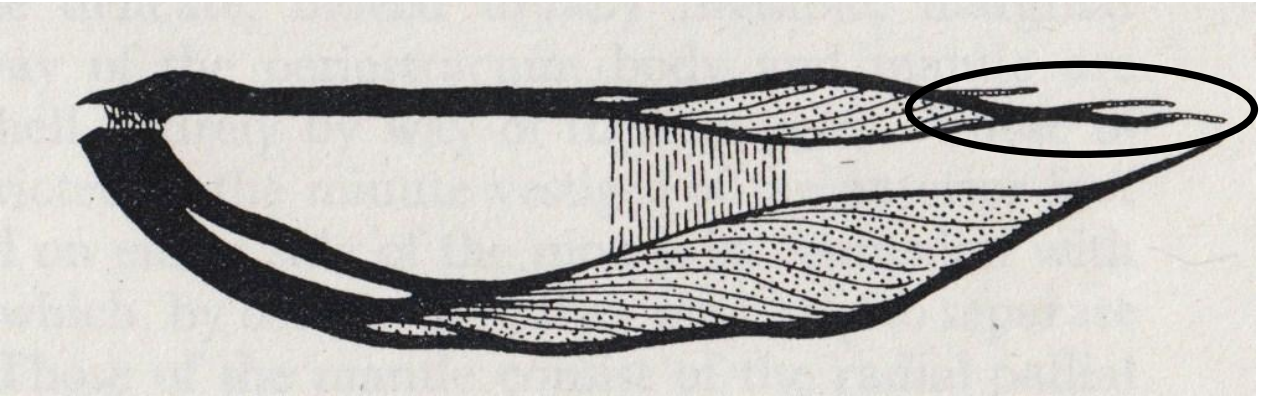


Figure 2-9: Oyster shell showing an outer layer called prismatic layer (circled) formed by calcite and located only in the right valve, which is not entirely rigid (source: Yonge, 1960).

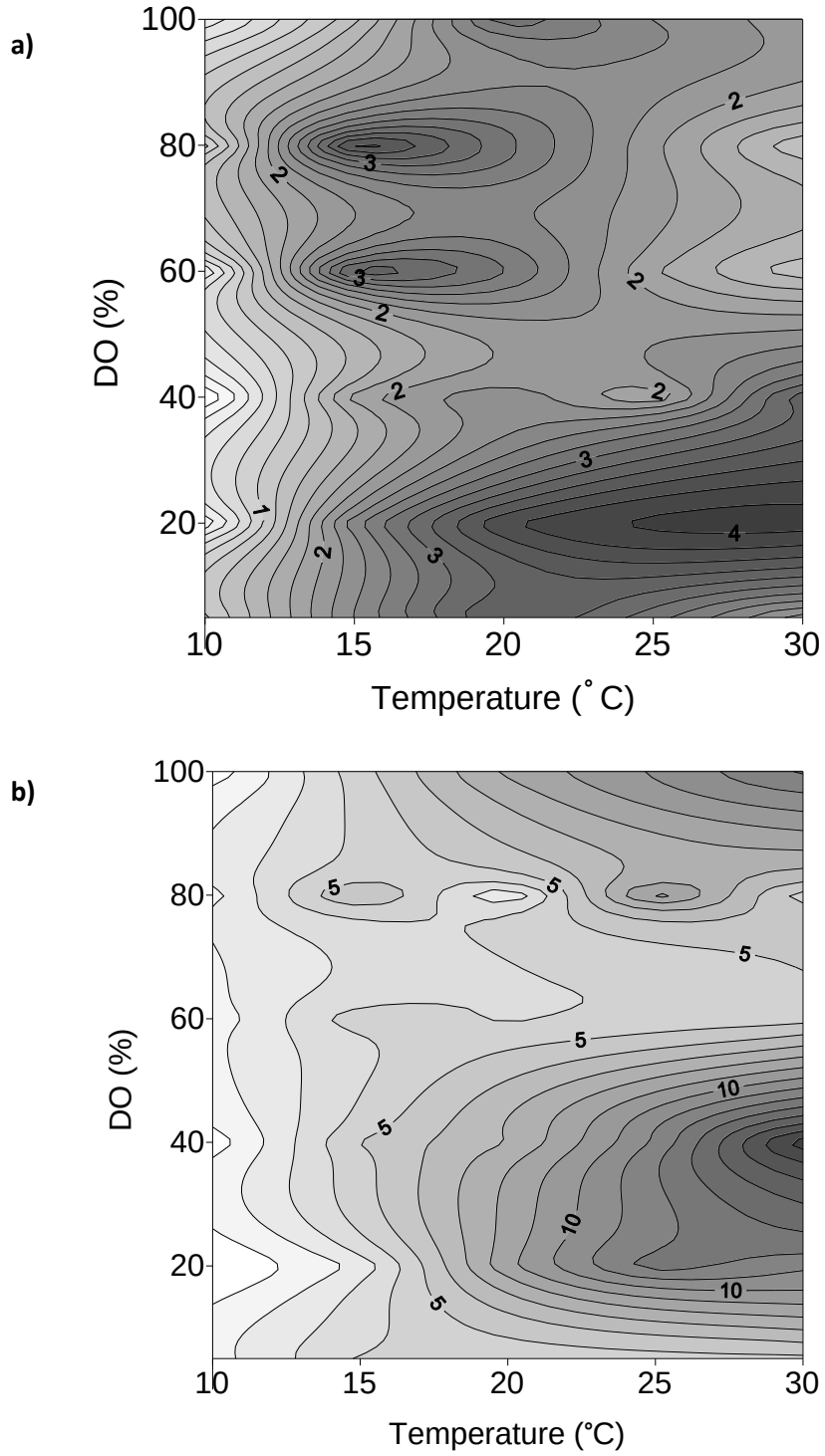


Figure 2-10: Distribution of **a)** mean valve gap and **b)** mean number of spikes with temperature (°C) and DO (%).

Table 2-1: Experiment conditions shown by average value of temperature (° C) and dissolved oxygen level (% and mg L^{-1}).

Intended DO level (%)	Temperature (°C)	s.d.	DO (%)	s.d.	DO (mg L^{-1})	s.d.	Intended DO level (%)	Temperature (°C)	s.d.	DO (%)	s.d.	DO (mg L^{-1})	s.d.
100%	10.1	0.2	97.7	0.5	9.1	0.0	40%	10.4	0.2	39.5	0.3	3.7	0.0
	14.9	0.2	98.1	0.6	8.2	0.0		15.1	0.3	39.9	0.2	3.3	0.0
	20.4	0.1	96.7	0.2	7.3	0.0		20.3	0.1	40.4	0.2	3.1	0.0
	24.1	0.1	96.5	0.4	6.8	0.0		24.5	0.4	40.4	0.2	2.8	0.0
	29.1	0.5	97.3	0.8	6.3	0.0		29.4	0.2	38.9	0.5	2.5	0.0
	19.2	0.2	97.3	0.9	7.5	0.1		20.4	0.2	40.3	0.3	3.0	0.0
80%	10.2	0.3	80.9	0.9	7.5	0.1	20%	10.7	0.2	19.1	0.2	1.8	0.0
	15.0	0.2	80.4	1.0	6.7	0.1		15.5	0.2	19.7	0.9	1.6	0.1
	20.5	0.1	78.9	0.2	6.0	0.0		19.5	0.1	19.3	1.3	1.5	0.1
	25.2	0.2	81.3	0.8	5.6	0.1		24.2	0.1	16.1	2.6	1.1	0.2
	29.9	0.4	80.6	0.4	5.2	0.1		29.8	0.4	19.6	0.7	1.3	0.0
	20.3	0.2	79.6	2.5	6.0	0.2		20.4	0.1	19.3	1.4	1.5	0.1
60%	10.4	0.2	60.4	0.3	5.6	0.0	5%	10.5	0.2	5.3	1.7	0.5	0.2
	15.8	0.3	60.0	1.6	5.0	0.1		15.2	0.2	4.3	0.3	0.4	0.0
	20.3	0.1	59.9	0.1	4.5	0.0		19.9	0.0	4.6	0.1	0.3	0.0
	25.2	0.2	60.3	0.2	4.2	0.0		25.2	0.2	5.3	0.2	0.4	0.0
	30.3	0.2	60.3	0.2	3.8	0.0		30.1	0.5	5.4	0.2	0.3	0.0
	20.4	0.2	60.2	1.7	4.5	0.1		20.4	0.1	4.6	0.2	0.3	0.0

Chapter 3.

Water quality improvement and fertilization in Omura Bay using an artificial upwelling

3.1 Introduction

Upwelling regions are among the most dynamic and important regions to boost primary production (Karp-Boss, 2004). Besides, the coastal ocean produces more than 90% of global fisheries which is directly related to the upwelling systems (Lindeboom, 2002). Concentration of nutrients in cold deep waters is usually higher than that in the surface. Upwelling areas are usually of high production because cold nutrient–rich deep water is advected upwards with the flow and mixing of water column. In natural upwelling areas, stable carbon and nitrogen isotope ratio of particulate organic matter have been used to trace biochemical processes, which are linked to variable nutrient supplies and origin of particulate organic matter (Liebes and Deuser, 1988; Wu *et al*., 1999; Walker and McCarthy, 2012).

The growth of world´s population and the impossibility of obtaining the future demanded seafood from natural stocks indicate a necessity of new technologies to increase ocean productivity. Based on the natural process concept, artificial upwelling is

now regarded as an important strategy to enhance of marine production potential and activities such as filter-feeding shellfish aquaculture.

In the central basin of Omura Bay, hypoxia is formed every summer in consequence of higher rates of sediment consumption (Wada et al., 2012), poor circulation forming a stagnant area (Takahashi, 2009) and water stratification. Previous studies have concluded that aeration from the bottom or dredge of the sediment would help reducing the formation of oxygen-deficient water mass and supposedly increase the production.

3.2 Objectives

This study investigates the efficiency of an artificial upwelling in improving water quality in a summer stratified bay, by inducing mixing as well as positively affecting biogeochemical processes such as primary production. This is important because improvements in water quality during summer poor conditions are required for sustaining ecosystems and supporting an important economical activity: the mariculture.

3.3 Materials and methods

Aeration cables connected to non-oil compressors (Hitachi oil free screw compressor) located inland and extending for 7 km on the bottom, towards the centre of Omura Bay, close to the known hypoxia formation start point (Fig. 3-1). Aeration from the sea-bottom was performed during 2 summer seasons, from 4 June until 30 September 2011, and from 25 May to 30 September 2012. Initially, air supply was planned to be at a rate of 1.26 m^3 min^{-1} for both years. However, in 2011, the aeration cable was buried in the bottom sediment which clogged the pipes, resulting in an aeration of 0.4 m^3 min^{-1}, or 1/3 of the expected aeration for that year. Therefore, data in 2012 are believed to properly show effects of the aeration, and data in 2011 were considered as control. Some selected 2011 data are shown for comparative discussion.

Two approaches were developed for field sampling: the first one covered Omura Bay extension as a whole in a diagonal length (Sts. 1, 2, 5, 10, 15, 17, 18 and 21). The second one concentrated in an area within 2 km from the aeration point inserted in the bigger diagonal line and consisted of a temperature monitoring mooring system (Sts. M1, M2, M3, M4, M5, from north to south). Aeration line was centred at St. M2 in 2011 and at St. M3 in 2012.

Temperature recorders (Tidbits, Onset Computer Co., USA) were installed in a mooring system measuring 80 and 160 meter-long in 2011 and 2012, respectively. Data were recorded at 10 min intervals at 5 different stations separated 20 m and 40 m apart in 2011 and 2012, respectively (see Fig. 3-2 for mooring system in 2012). The region

chosen for the installation has a 20-meter-deep water column and in each vertical line 5 sensors were attached at approximately depths of: 3 m, 7 m, 11 m, 15 m and 19 m (Fig. 3-2). Temperature sensor data were collected from the first day of aeration 4 June, until the end of experiment on 30 October 2011, and from 25 May until 30 October in 2012.

Temperature, salinity, dissolved oxygen concentrations and *in situ* fluorescence data were collected monthly every 0.1 m depth with a CTD (Rinko AAQ125 JFE Advantech) at predefined stations along a diagonal axis covering the whole bay, including stations within the mooring system (Figs. 3-1 and 3-2). TidBit recorders were calibrated for temperature data correction. Stratification index was calculated as the temperature difference between 3 m depth and 15 m depth at each station for the whole observation period, using the data from temperature recorders.

Water samplings were taken for nutrient concentrations (NO_3^-, NO_2^- PO_4^-), chlorophyll *a* (Chl *a*) concentration and carbon and nitrogen isotopes. Nitrate and nitrite forms of dissolved inorganic nitrogen (NO_3^- and NO_2^-), phosphate and silicate concentrations were measured using the QuAATro 2MR Auto Analyzer (BL-Tec). Chlorophyll *a* was extracted from particulate organic matter on the GF/F filter in the dark for 12 h by 90% acetone, and the concentration was measured using a calibrated fluorometer (Trilogy Laboratory Fluorometer P/N 998-7210). Extracted Chl *a* concentrations and *in situ* fluorescence showed a good correlation ($R^2 > 0.80$), and thus fluorescence was calibrated to the Chl *a* concentrations (µg L^{-1}). Water samples were filtered through precombusted Whatman GF/F filters for elemental and isotopic analyses. Filter samples were put in desiccators with HCl flumes overnight to remove inorganic carbon, and then with NaOH pellets for 24 h to completely remove acid

vapour and CO_2. Each filter was folded and wrapped with a tin capsule. Particulate organic carbon (POC) and nitrogen (PN) concentrations, as well as carbon ($\delta^{13}C_{POC,}$ from now on cited as $\delta^{13}C$) and nitrogen isotopic ratios ($\delta^{15}N_{PN,}$ from now on cited as $\delta^{15}N$) were measured using a mass spectrometer coupled with an elemental analyzer (EA Conflo IV+DELTA V Plus) and expressed as per mil (‰) deviation from the standard defined by the following equation:

$$\delta^{13}C\ (‰) \text{ or } \delta^{15}N\ (‰) = (R_{sample} / R_{standard} - 1) \times 10^{3},$$

where R is $^{13}C/\ ^{12}C$ or $^{15}N/\ ^{14}N$ and the standard is either the Pee Dee Belemnite or atmospheric nitrogen, respectively.

3.4 Results

There was a seasonal distribution in hydrographic parameters (temperature and salinity) and hypoxia formation in the Bay, which can be observed in the 2012 vertical sections. In May 2012, water column in the inner bay was already stratified with differences of 4 °C between surface and bottom layers, but hypoxic water was not present (Fig. 3-3). The formation of hypoxic water started in June, and oxygen-depleted water covered almost the whole bottom extension of the inner bay (see Fig. 1-2 for bay regions) in July. The stratification reached its peak in July (approximately 10 °C difference between surface and bottom layers) (Figs. 3-4 and 3-5). In August, hypoxic water extension reached its peak, but later in September the extension of hypoxic water

was smaller and localized (Figs. 3-6 and 3-7). Colder water was observed in the bottom layer, at the same location where the hypoxic water was observed. Chlorophyll *a* blooms were formed on the area surrounding the aeration spot in June, July, September and October, at different depths, but were not formed in other areas of Omura Bay extension. In the beginning of October there was a horizontal salinity gradient, oriented from the bay mouth towards the inner bay (Fig. 3-8, see Fig. 1-2 for bay regions). Aeration pushed water towards surface, as indicated by the isolines in the vertical sections of the whole bay extension (St. M3 located at around 17 km, Figs. 3-3 to 3-8) and at the focused aeration vertical sections (St. M3 located at around 880 m, Figs. 3-9 to 3-11).

Vertical profiles of stations in the mooring system in 2011 did not show differences that could have been caused by the aeration in the water column characteristics among stations (Figs. 3-12 and 3-13), apart from an increase in chlorophyll a concentration at St. M2 in October, when thermal stratification was low. However, in 2012 water column at the mooring system stations showed changes in temperature, chlorophyll *a* and DO induced by the artificial upwelling. Temperature and salinity at Sts. M1 and M3 just after the beginning of the aeration (June and July) were similar to those at Sts. 21 and 22 (Fig 3-14). In August and September whole water column at Sts. M1 and M3 was relatively colder than at Sts. 21 and 22. Water column at Sts. M1 and M3 was more thermally homogeneous, compared to St. 21 (Fig. 3-15), and that also extended to the October sampling, just after the aeration had been stopped (Fig. 3-16). From July to October, chlorophyll *a* concentration at Sts. M1 and M3 was significantly higher than Sts. 21 and 22, initially mainly on the bottom layers (deeper

than 15m depth; Figs. 3-14 and 3-15) and then in October, especially at the surface layers (Fig. 3-16). Dissolved oxygen concentration at stations near the aeration were higher than at Sts. 21 and 22, initially above 15 m depth in June and in whole water column in July, respectively (Fig. 3-14). Dissolved oxygen concentration was also increased at the bottom layers in the beginning of October at the stations where the aeration had been performed (Fig. 3-16).

Tidbit data showed that the temperatures decreased with depth and that the stratification of the water column persisted until the middle of September in both years. Stratification indexes in 2011 were similar among stations showing water column along the mooring system shared same characteristics (Fig. 3-17). In 2012 however, differences in temperature were observed among stations (Fig. 3-18). Bottom layers at all mooring stations, between approximately 11 and 15 m were mixed. However, at St. M2 water was thermally mixed on a broader depth range, between 7 and 15 m. Water column was less stratified at St. M2 than the others (Fig. 3-18). Unfortunately, the Tidbits at St. M3 had a malfunctioning and data was not recorded at all depths.

Nitrate, nitrite and phosphate generally increased with depth in summer, especially in 2011 (Fig. 3-19). However, in 2012, a different tendency was shown among stations although not for all nutrients. Nitrate in August and October, nitrite in August, September and October and phosphate in October had a pattern of higher and lower concentration at surface and bottom layers respectively, at St. M3, when compared to the other stations (Fig. 3-20).

Isotopes for both carbon and nitrogen showed small variations among stations in the aeration phase. In 2011 it was not observed a clear tendency on isotopic data (Fig. 3-21). Carbon to nitrogen ratios were high and similar among stations (average at Sts. 21, 22 and M2 were 8.53, 9.35 and 8.79, respectively; not shown). In 2012, carbon and nitrogen isotopes values were ranged from -29.2 to -17.2, and 4.7 to 9.7, respectively and did not show a distinct pattern among Sts. 21, 22 and M3 (Kruskal-Wallis, $p = 0.777$ and $p = 0.247$, for carbon and nitrogen isotopes, respectively). Nevertheless, carbon isotopes were slightly enriched at St. M3, compared to the others (Fig. 3-22). Carbon to nitrogen ratios were generally bellow Redfield ratio of 6.7 at St. M3, whereas Sts. 21 and 22 had higher values, ranging from 5.9 to 9.9 (Fig. 3-23) and showed distinct tendency among stations, with lower values near the aeration, although it was statistically insignificant (Kruskal-Wallis, $p = 0.06$). During the aeration period, carbon to chlorophyll ratios were generally lower at St. M3 than at the other stations, especially in the bottom and middle layers (Table 3-1).

3.5 Discussion

To closely investigate the effects of an artificial upwelling that was installed on the bottom of Omura bay, oceanographic samplings were performed covering a diagonal line in the Bay extension and temperature recorders were installed in the mooring system close to the aeration centre. Aeration was weak in 2011, and showed no significant changes to water characteristics that could have been induced by the aeration.

In 2012, on the other hand, it was possible to identify small changes induced by the artificial aeration. Water column at stations near the aeration (Sts. M1 and M3) showed low temperature and higher mixing. Initially, the water was colder at Sts. M1 and M3 in the upper layers and then with time the aeration decreased the water temperature in deeper depths, getting colder than Sts. 21 and 22 (Figs. 3-14 and 3-15). The same trend was observed for the increase of DO levels, which was identified on surface layers at Sts. M1 and M3 in the first months, before the summer temperature peak. Later, in the beginning of autumn, DO increased in the lower depths at those stations. Chlorophyll *a* blooms were observed especially in the bottom layers at St. M1 and M3, as a result of upwelled nutrients.

In August and September 2012, the aeration could not maintain high oxygenation levels, whereas temperature was maintained cooler. With the decay of water stratification in September, hypoxic water retracted due to mixing (Figs. 3-7 and 3-15). In the beginning of October, vertical stratification was destroyed but a horizontal gradient was formed due to the intrusion of more saline water from the straits. This induced mixing of the bay water and marked the end of summer stratification period in the bay. Temperature at stations near the aeration remained low and chlorophyll *a* in the surface layers and oxygen levels were improved due to residual effects of aeration (Figs. 3-8 and 3-16). In the end of October Sts. 21 and 22 water column is homogeneous, while St. M1 and M3 are stratified due to an intrusion of water from the strait towards the inner bay, through the bottom and middle layers.

Elevated chlorophyll *a* concentration and $\delta^{13}C$ of POM in the surface waters are most likely the result of the response of phytoplankton to upwelled nutrient-rich

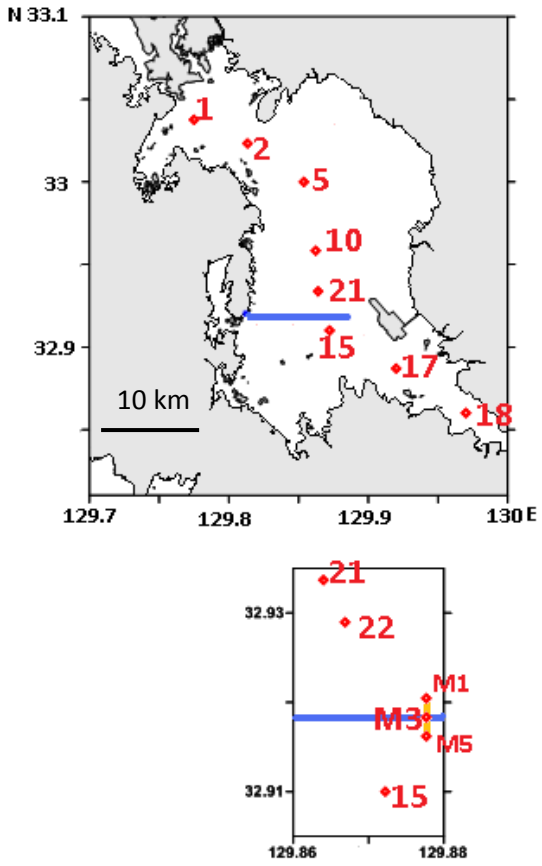
water in 2012. However, nutrient concentrations were similar at all stations in June and July of that year. From August 2012, St. M3 showed lower dissolved nitrogen concentration in the water column. The uptake by the phytoplankton could be the reason for these slight differences in concentrations. Dilution may have induced the lower concentration of nutrients in the bottom layers of St. M3. Nutrient concentration increased in the upper layer at the expense of the upwelling which induced dilution due to mixing of the nutrients in bottom layer.

The difference in mean C:N and C:Chl a ratios of POM between St. M3 and the other stations in 2012, with lower values at the first than at the latter, suggests that chlorophyll-containing POM might be associated with higher production induced by nutrients brought up by the upwelling. POM at St. M3 was more enriched with nitrogen (mean C:N ratio = 6.8 ± 0.7) compared to St. 21 (mean C:N ratio = 7.7 ± 0.9). Higher C:N values were observed at St. 21, which could be due to a decoupling between nitrogen and carbon dynamics at St. 21 as a result of nitrate depletion. Recent work has shown similar results in a natural upwelling region in a continental shelf south of Carmel, California (Walker and McCarthy, 2012). They thought the trends observed in C:N ratios were consistent with N-rich POM during upwelling events and more C-rich POM persisting during periods of water stratification. The differences between Sts. M3 and others can be attributed to the mixing induced by the artificial upwelling system.

3.6 Conclusions

This chapter focused on investigating changes in summer stratification and formation of hypoxia promoted by an artificial upwelling system installed in the centre of Omura Bay, where hypoxic water mass formation is known to start. While it is clear that aeration was not significant to the change in environmental conditions in 2011, positive effects of the artificial aeration could be identified in 2012. Even like this, water mixing was much localized even in 2012, when the aeration was in accordance to the planned rate. Lower temperature and increased DO were only induced in the beginning and end of summer, therefore the aeration was not strong enough for the scale and depth of the bay. Despite the unclear results in stable isotopes, low C:N and C:Chl a ratios of the POM samples showed upwelling might be an important tool to promote redistribution and increase in nutrients. This supposition is supported by the increases in Chl *a* observed at Sts. M1 and M3 compared to the stations far from the aeration system. These improvements are important, because they might have a positive effect on oysters if performed at farming sites.

a)



b)

Figure 3-1: Location of: **a)** aeration line (blue line) in Omura Bay, the diagonal sampling stations and the mooring system location (yellow line) and **b)** station of the mooring system (from M1-northern tip, to M5 -southern end and M3 - aeration centre) and details of spacing between them. The stations were located with a span of 20 m or 40 m in 2011, or 2012, respectively.

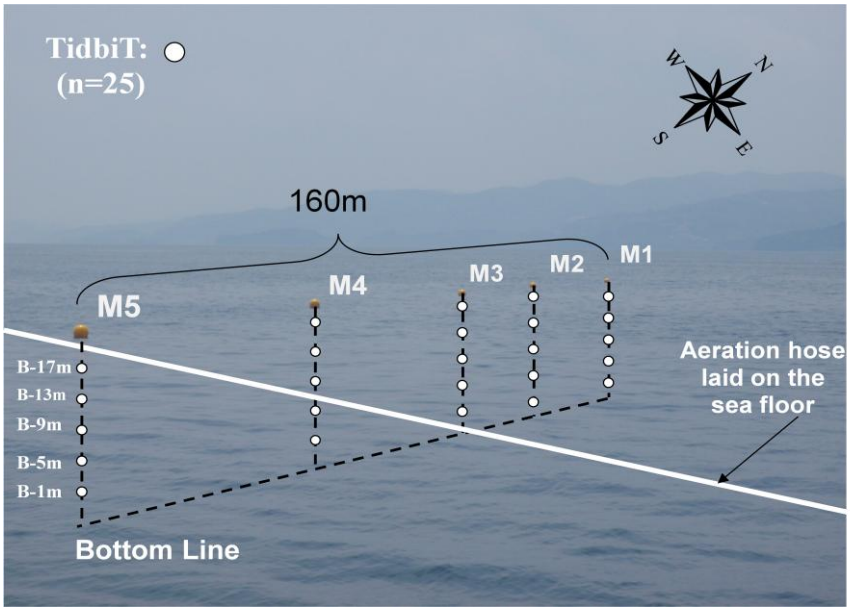


Figure 3-2: Scheme of the mooring system installed between St. 21 and St.15 in 2012, consisting of 5 stations with temperature recorders installed in 5 different depths. St. M3 is the aeration centre. Bottom ("B") depth in the region is approximately 20 m.

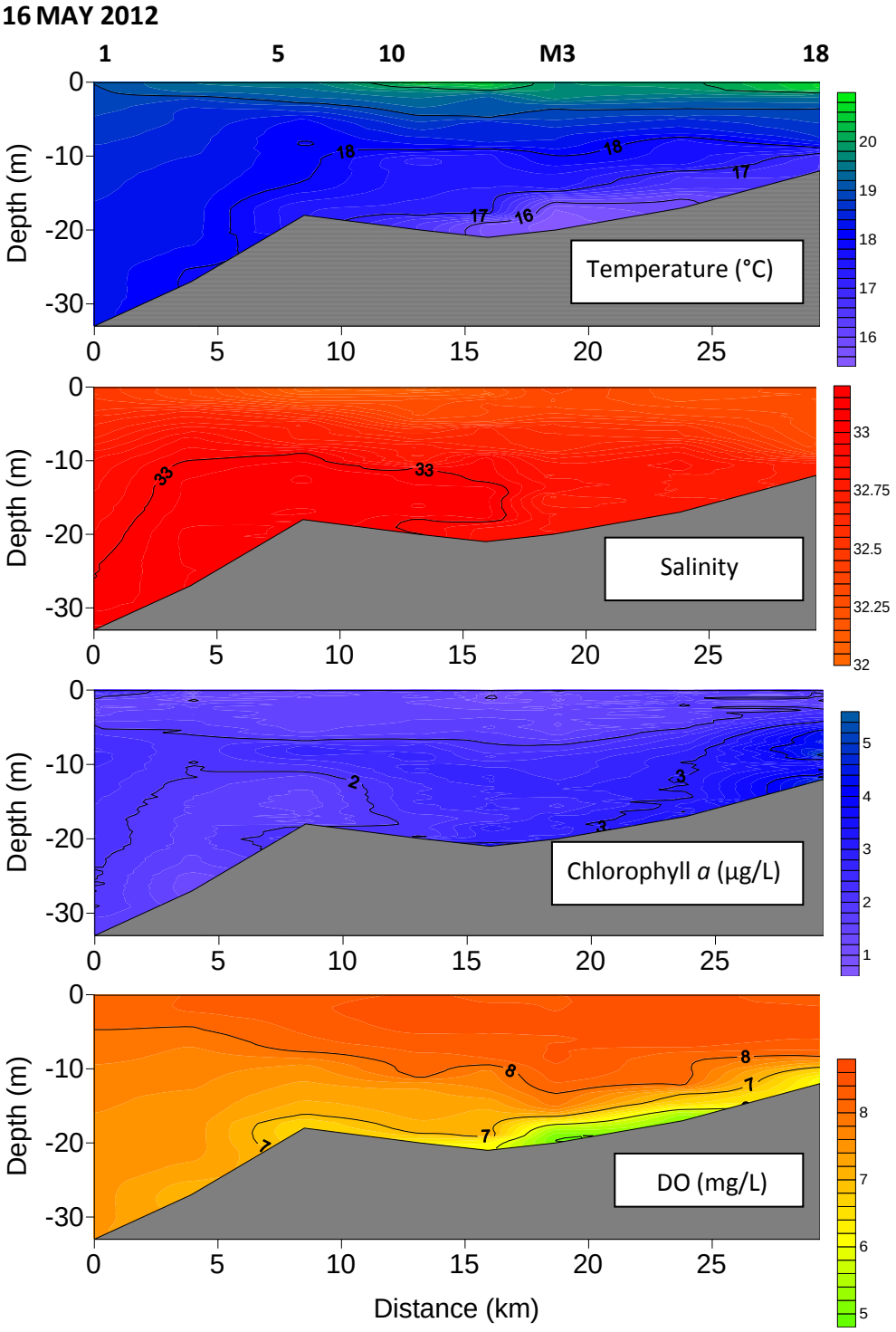


Figure 3-3: Vertical sections of temperature, salinity, chlorophyll *a* and dissolved oxygen for the diagonal bay sampling on 16 May 2012. Aeration was not performed in this sampling. Numbers above top vertical section indicate some referential stations.

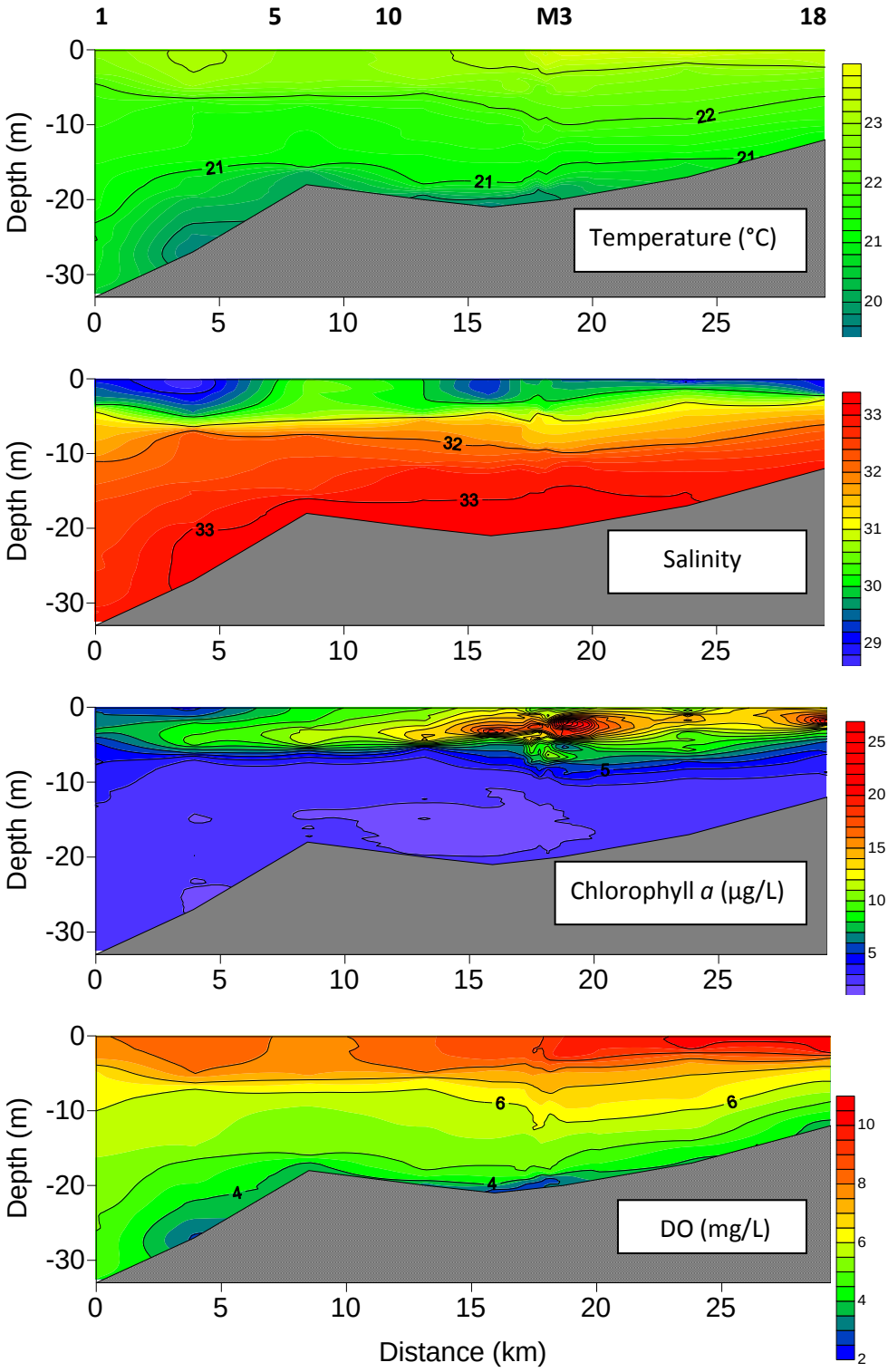


Figure 3-4: Vertical sections of temperature, salinity, chlorophyll *a* and dissolved oxygen for the diagonal bay sampling on 28 June 2012. Numbers above top vertical section indicate some referential stations.

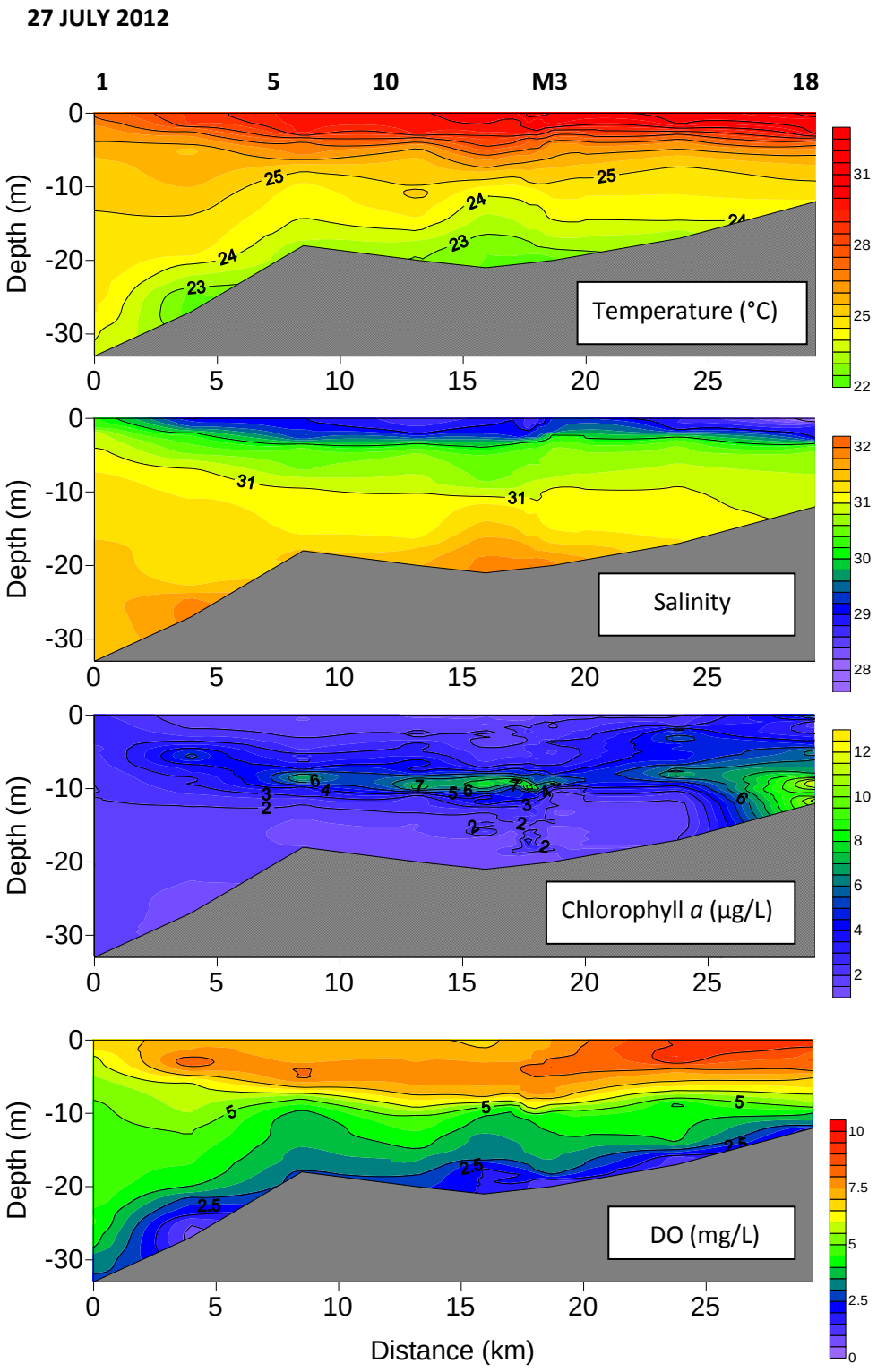


Figure 3-5: Vertical sections of temperature, salinity, chlorophyll *a* and dissolved oxygen for the diagonal bay sampling on 27 July 2012. Numbers above top vertical section indicate referential stations.

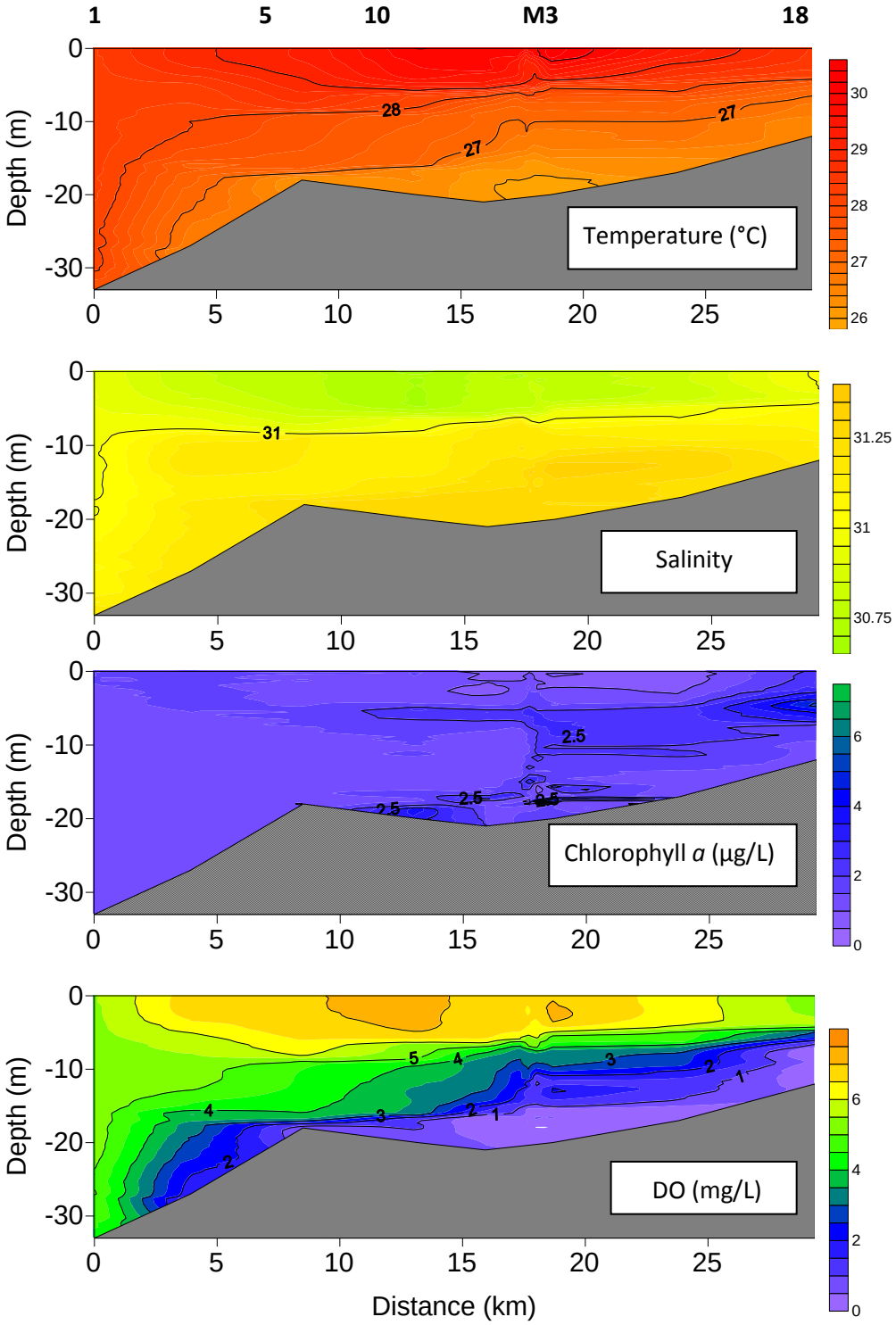


Figure 3-6: Vertical sections of temperature, salinity, chlorophyll *a* and dissolved oxygen for the diagonal bay sampling on 22 August 2012. Numbers above top vertical section indicate some referential stations.

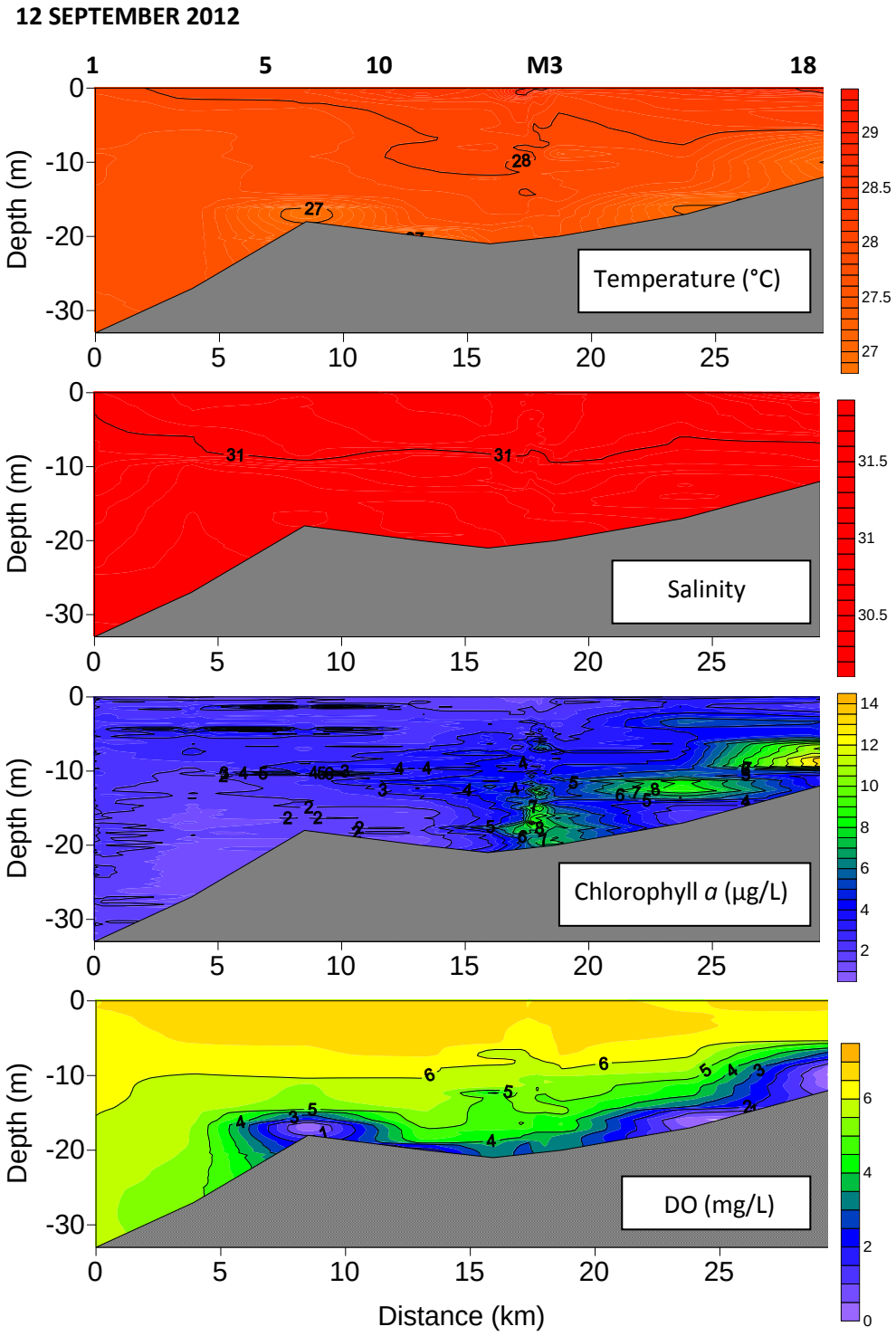


Figure 3-7: Vertical sections of temperature, salinity, chlorophyll *a* and dissolved oxygen for the diagonal bay sampling on 12 September 2012. Numbers above top vertical section indicate some referential stations.

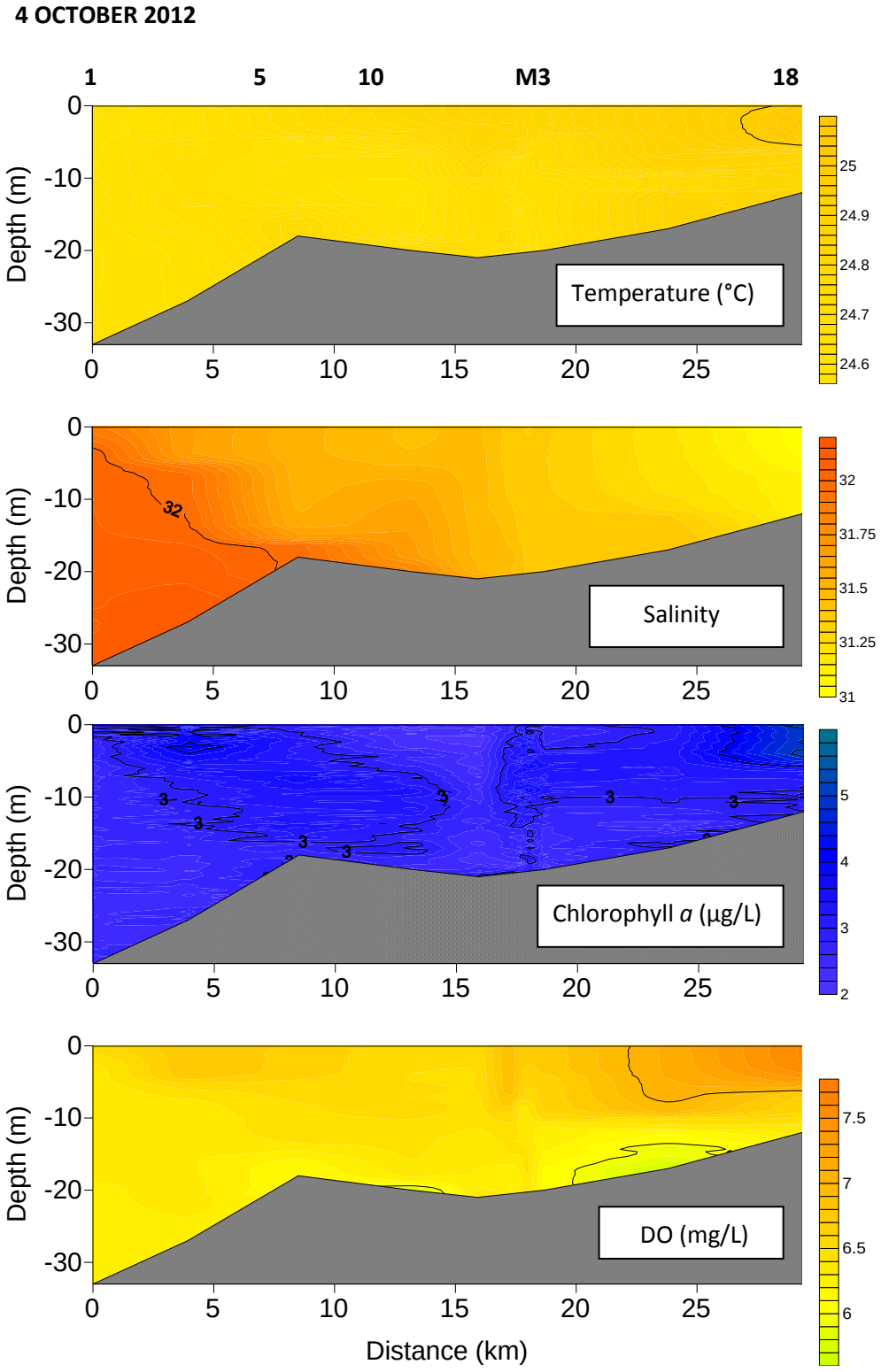


Figure 3-8: Vertical sections of temperature, salinity, chlorophyll *a* and dissolved oxygen for the diagonal bay sampling on 4 October 2012. Numbers above top vertical section indicate some referential stations.

28 JUNE 2012 **27 JULY 2012**

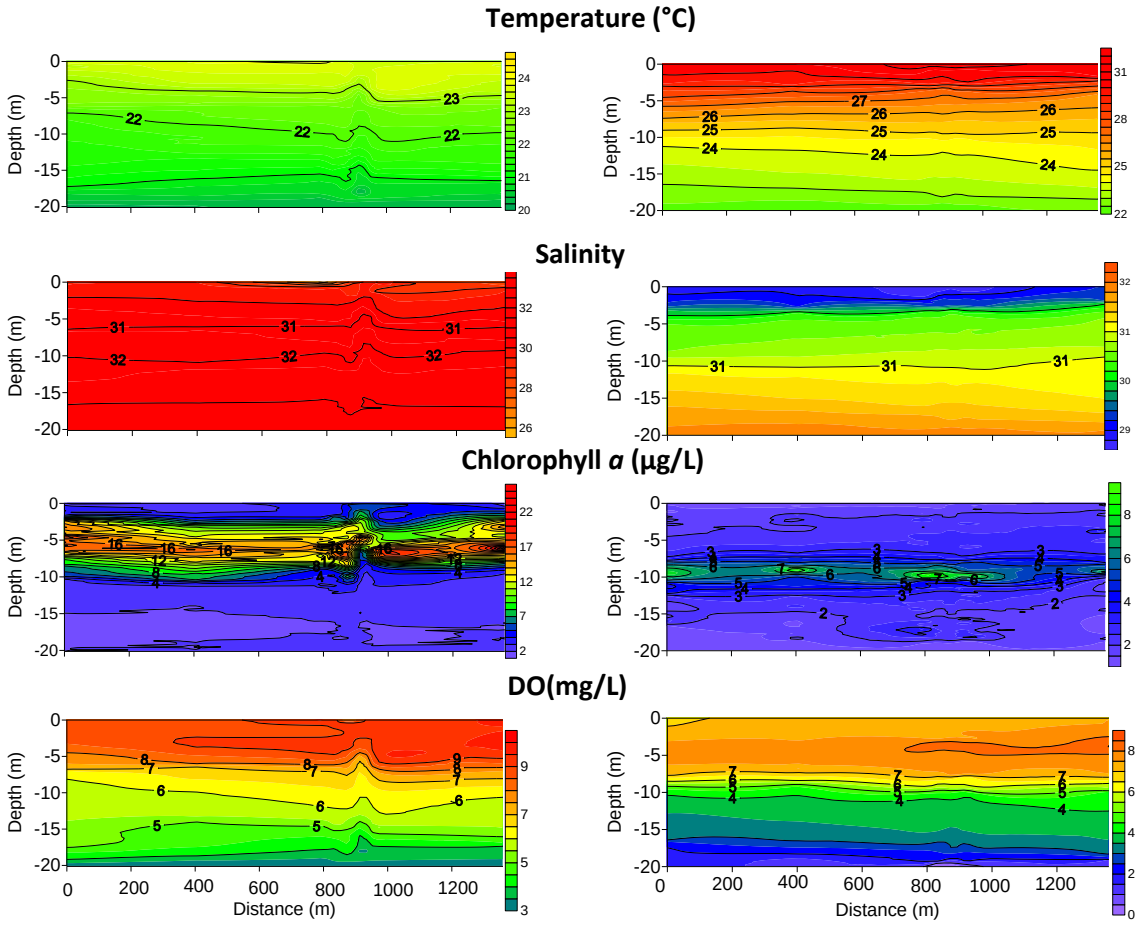


Figure 3-9: Vertical sections of temperature, salinity, chlorophyll *a* and dissolved oxygen along the mooring system on 28 June and 27 July October 2012. Aeration is located at approximately 880 m.

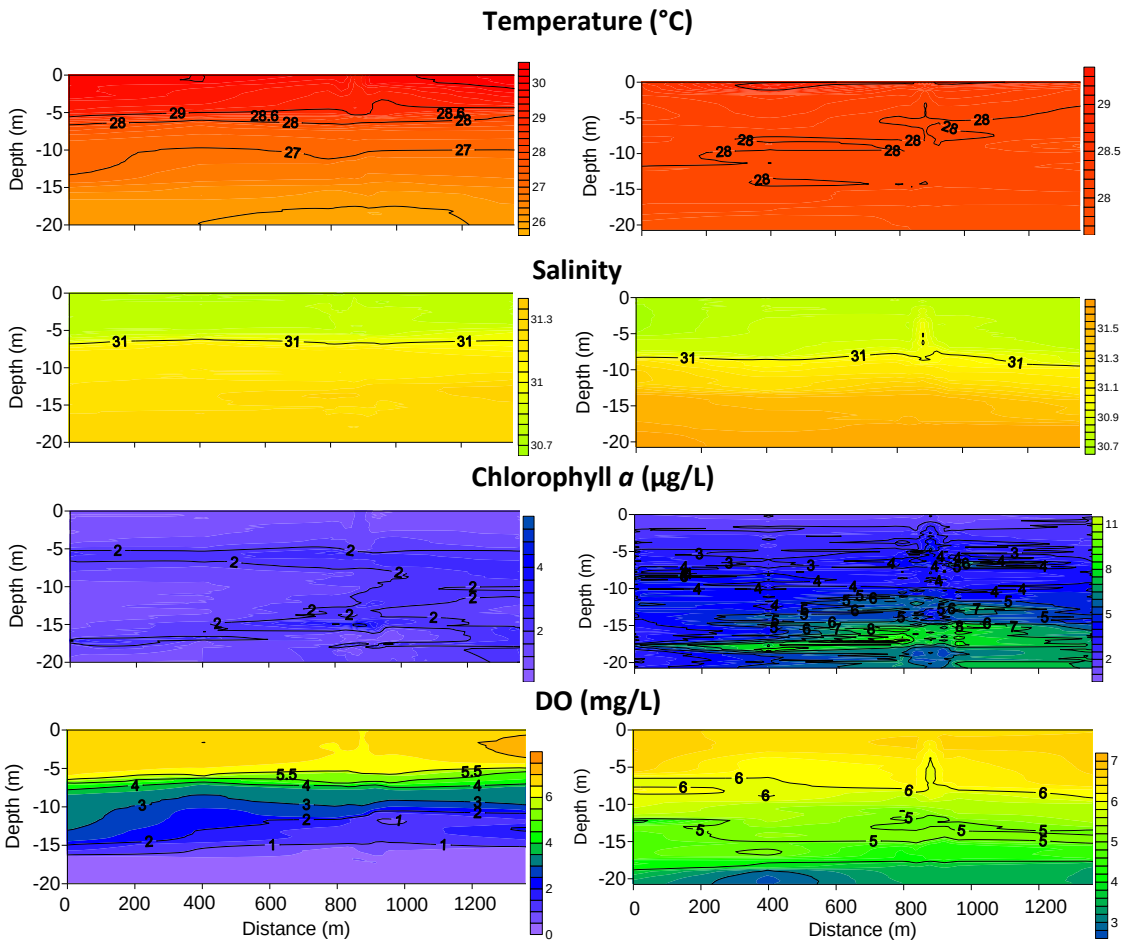


Figure 3-10: Vertical sections of temperature, salinity, chlorophyll *a* and dissolved oxygen along the mooring system on 22 August and 12 September 2012. Aeration is located at approximately 880 m.

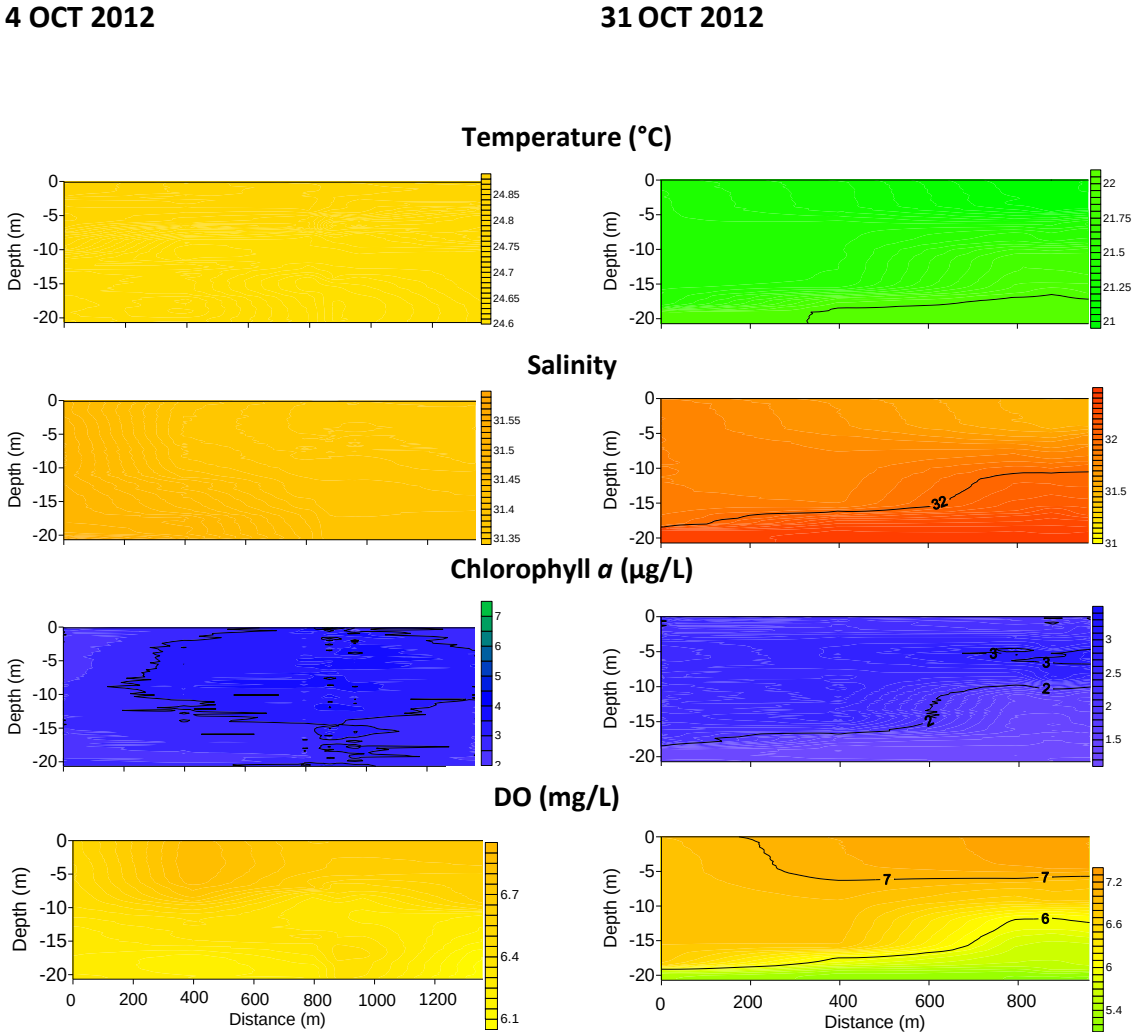


Figure 3-11: Vertical sections of temperature, salinity, chlorophyll *a* and dissolved oxygen along the mooring system on 4 and 31 October 2012. Aeration is located at approximately 880 m. In the last sampling (31 October) vertical section does not include St. 15.

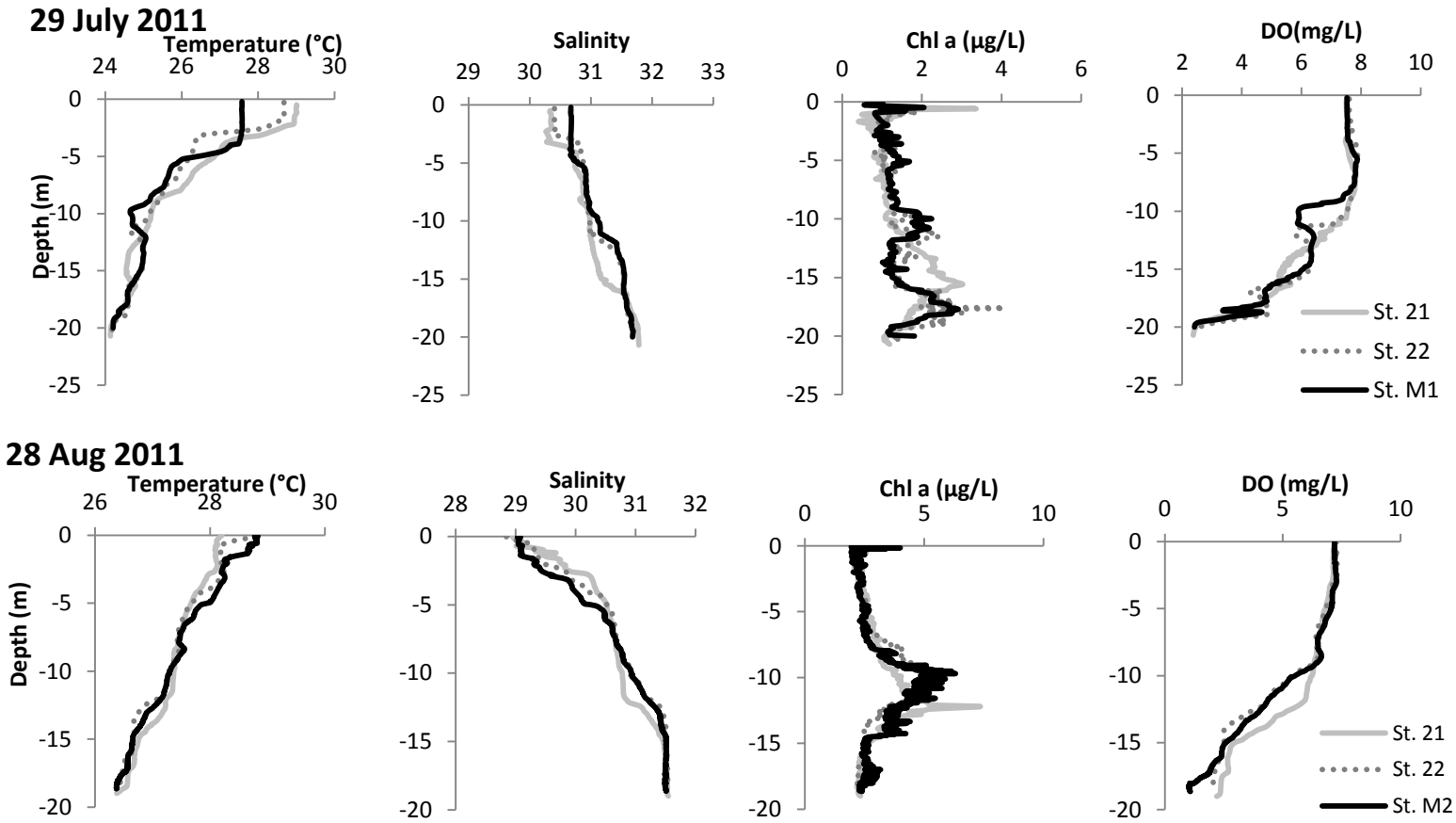


Figure 3-12: Vertical profiles of temperature, salinity, chlorophyll a (µg L^{-1}) and dissolved oxygen (mg L^{-1}) at Sts. 21, 22, M1 and M2 in July and August in 2011.

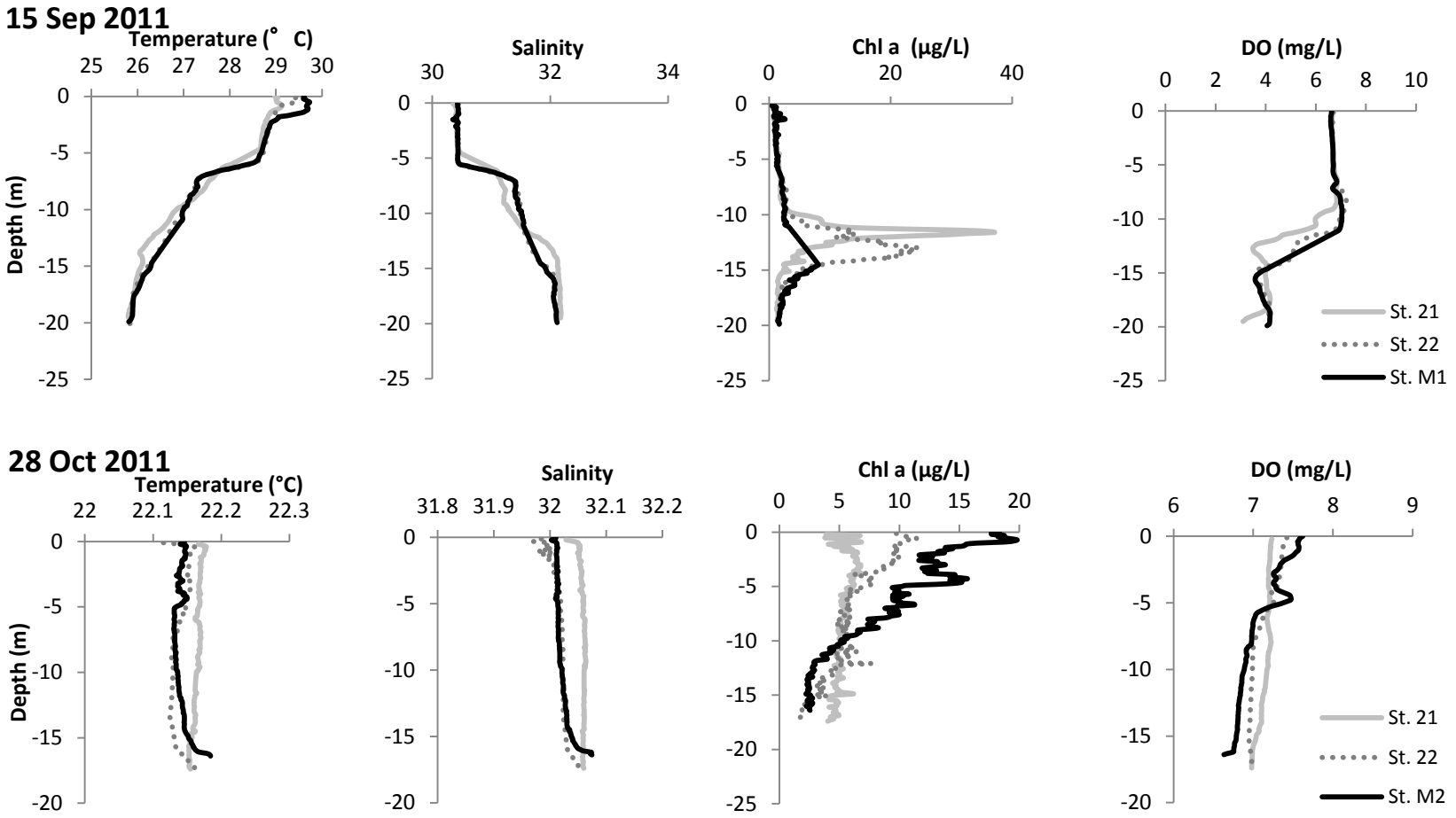


Figure 3-13: Vertical profiles of temperature, salinity, chlorophyll a (µg L^{-1}) and dissolved oxygen (mg L^{-1}) at Sts. 21, 22, M1 and M2 in September and October in 2011.

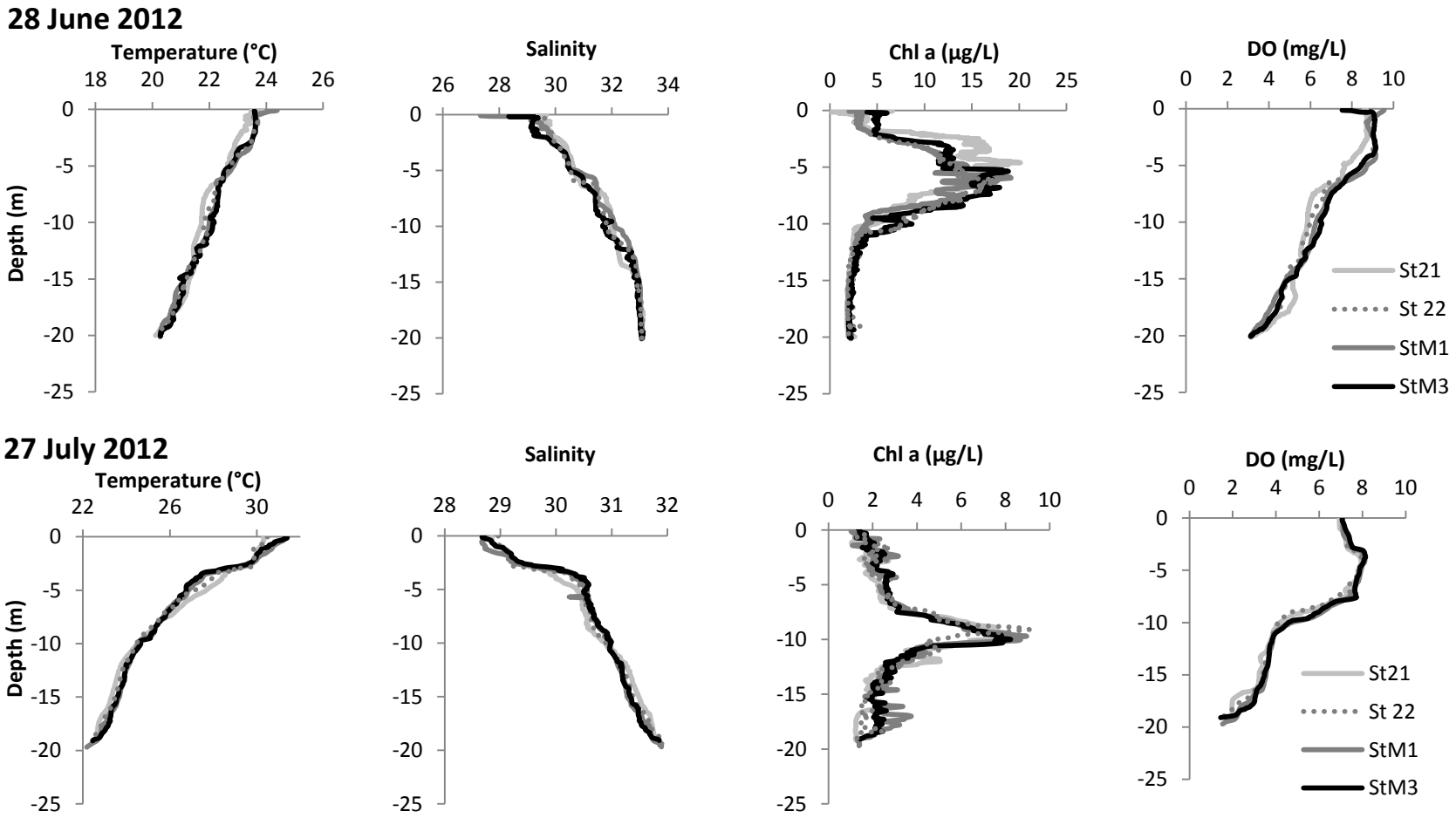


Figure 3-14: Vertical profiles of temperature, salinity, chlorophyll a (µg L^{-1}) and dissolved oxygen (mg L^{-1}) at Sts. 21, 22, M1 and M3 in June and July in 2012.

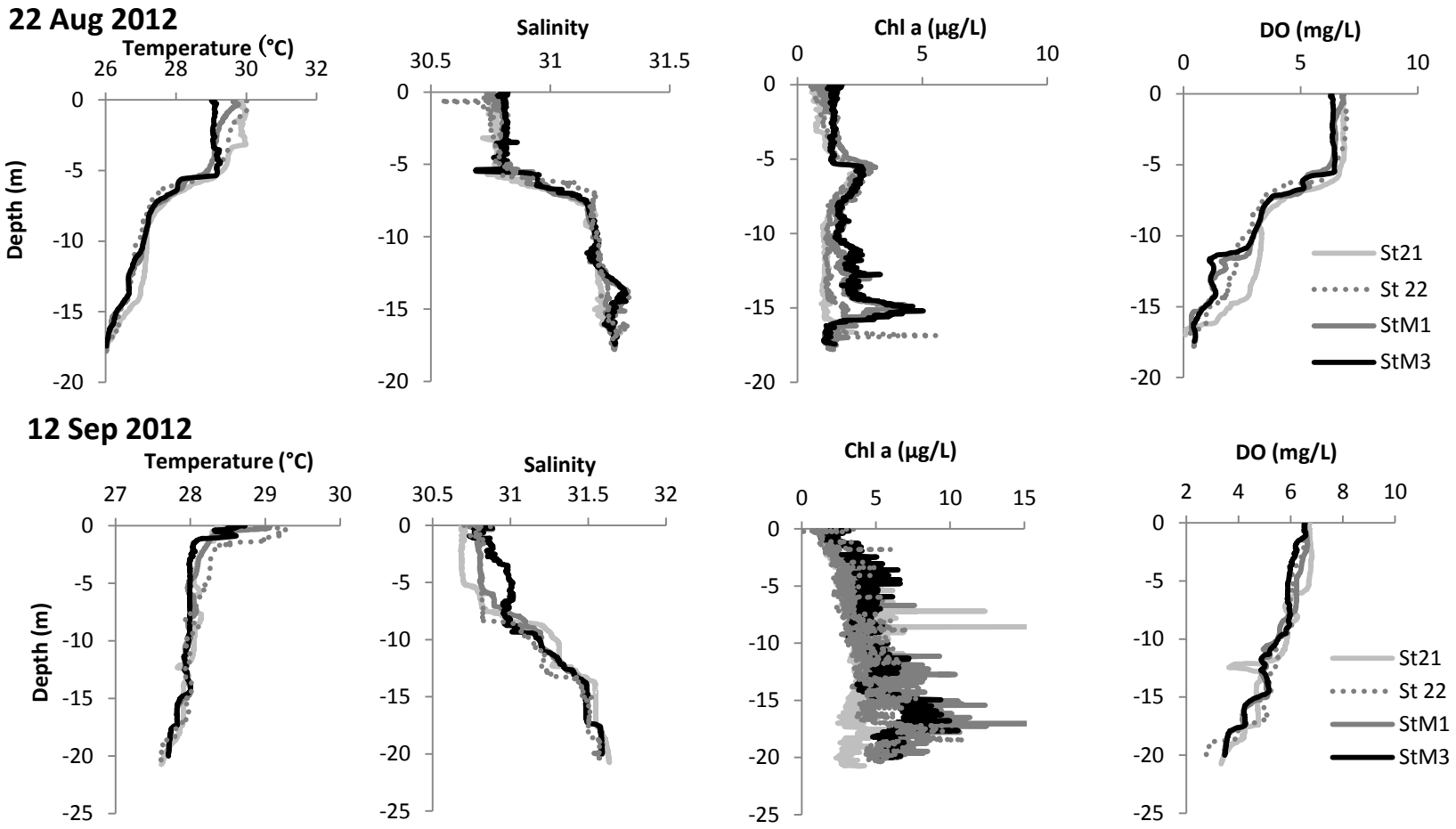


Figure 3-15: Vertical profiles of temperature, salinity, chlorophyll a (µg L^{-1}) and dissolved oxygen (mg L^{-1}) at Sts. 21, 22, M1 and M3 in August and September in 2012.

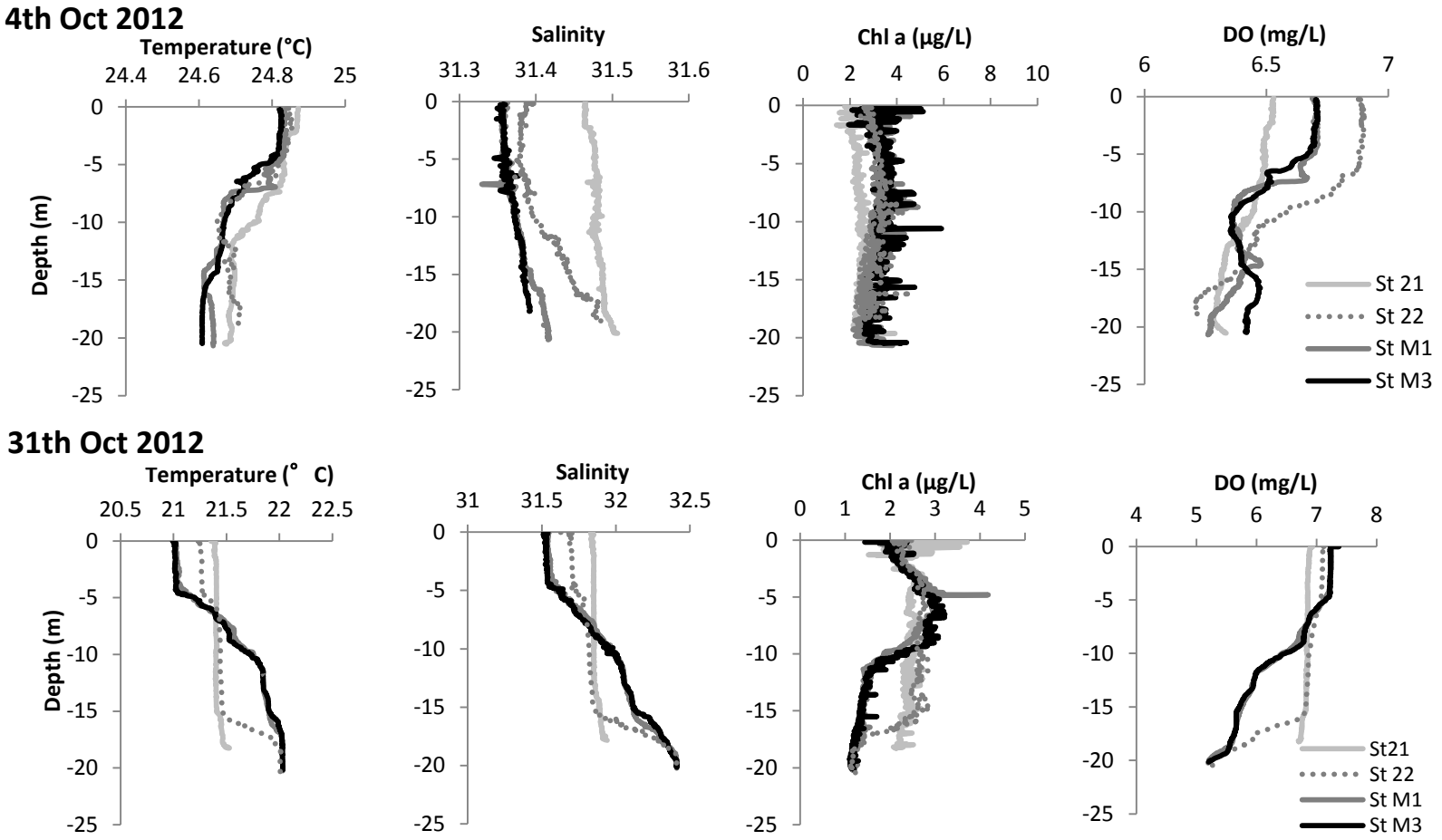


Figure 3-16: Vertical profiles of temperature, salinity, chlorophyll a (µg L^{-1}) and dissolved oxygen (mg L^{-1}) at stations 21, 22, M1 and M3 in the beginning and end of October in 2012.

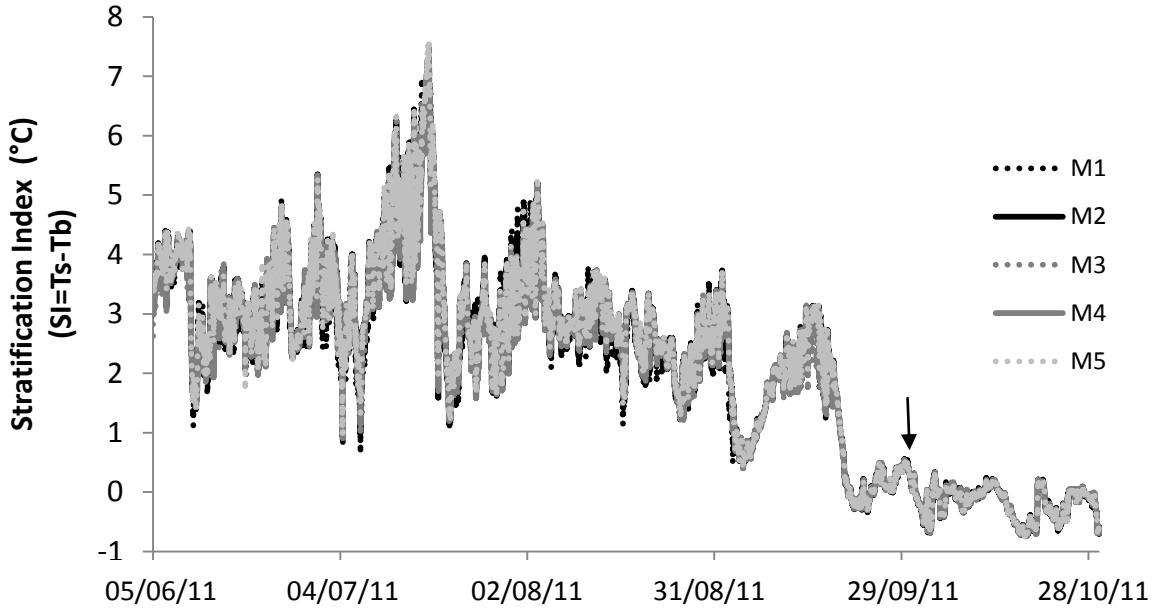


Figure 3-17: Stratification index at stations of the mooring system in 2011. Stations are located 20 m from each other and St. M2 is the centre of the aeration. Arrow indicates aeration end, on 30 September.

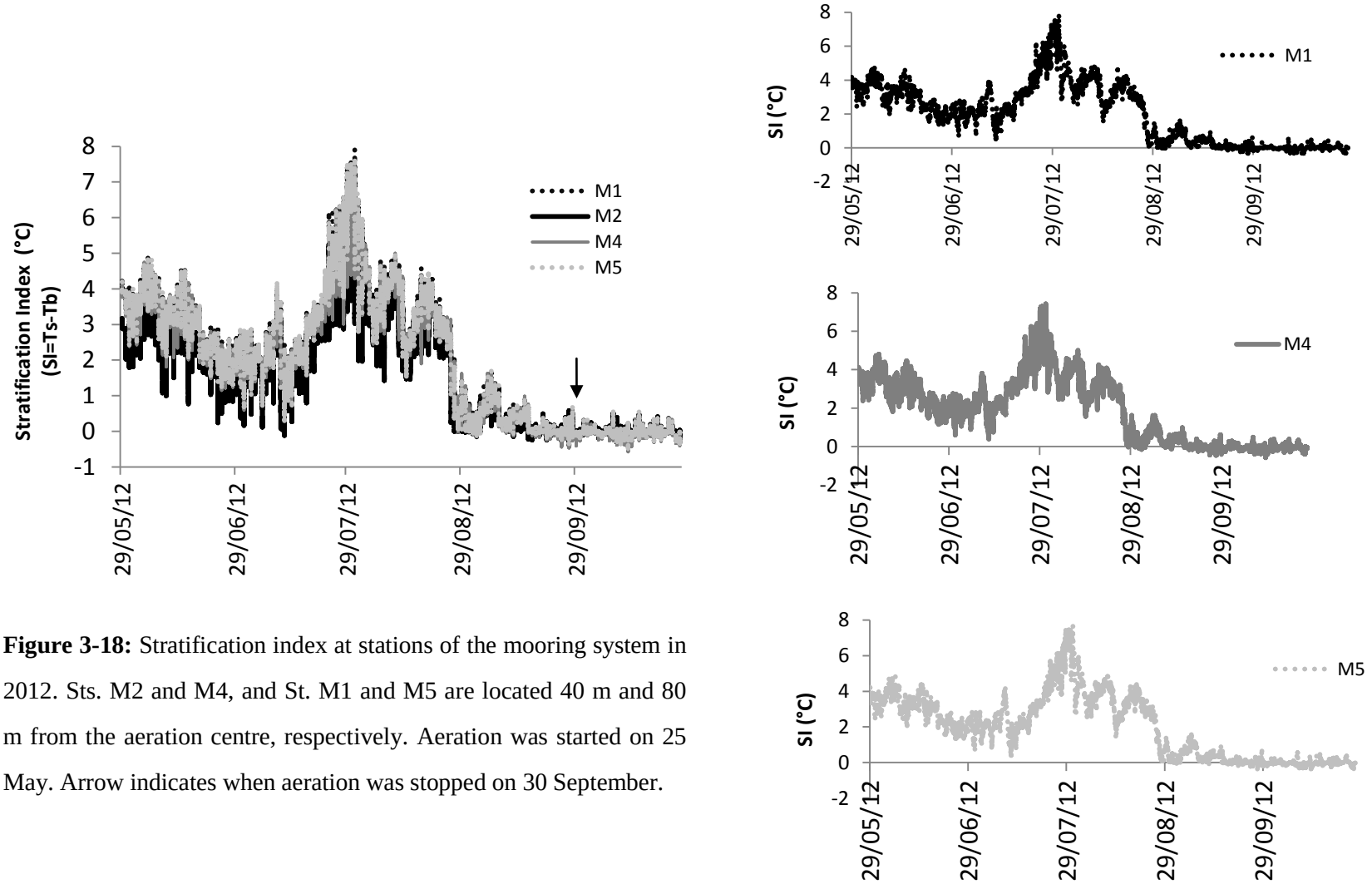


Figure 3-18: Stratification index at stations of the mooring system in 2012. Sts. M2 and M4, and St. M1 and M5 are located 40 m and 80 m from the aeration centre, respectively. Aeration was started on 25 May. Arrow indicates when aeration was stopped on 30 September.

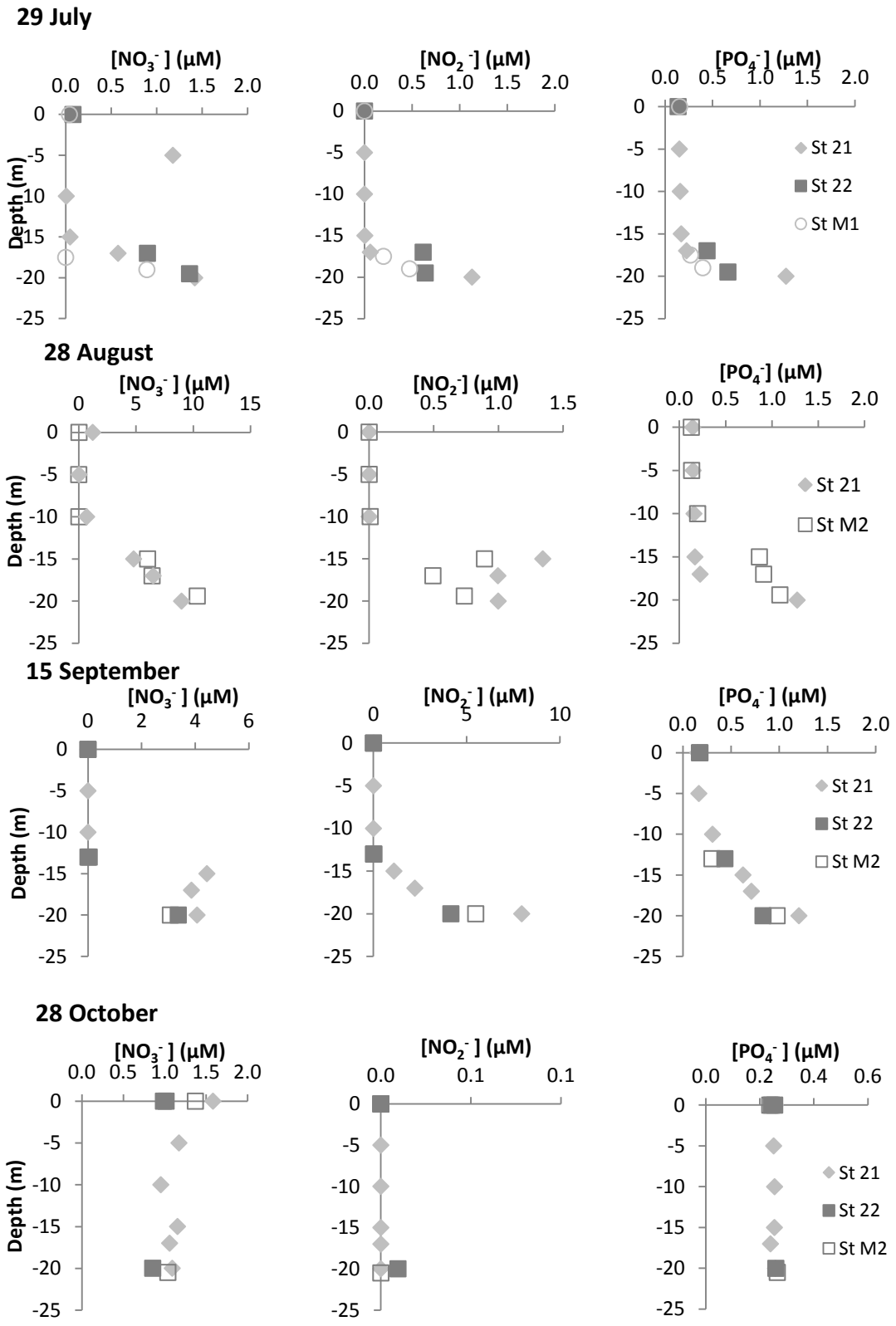


Figure 3-19: Vertical profiles of nitrate, nitrite and phosphate concentration (µM) in July, August, September and October 2011.

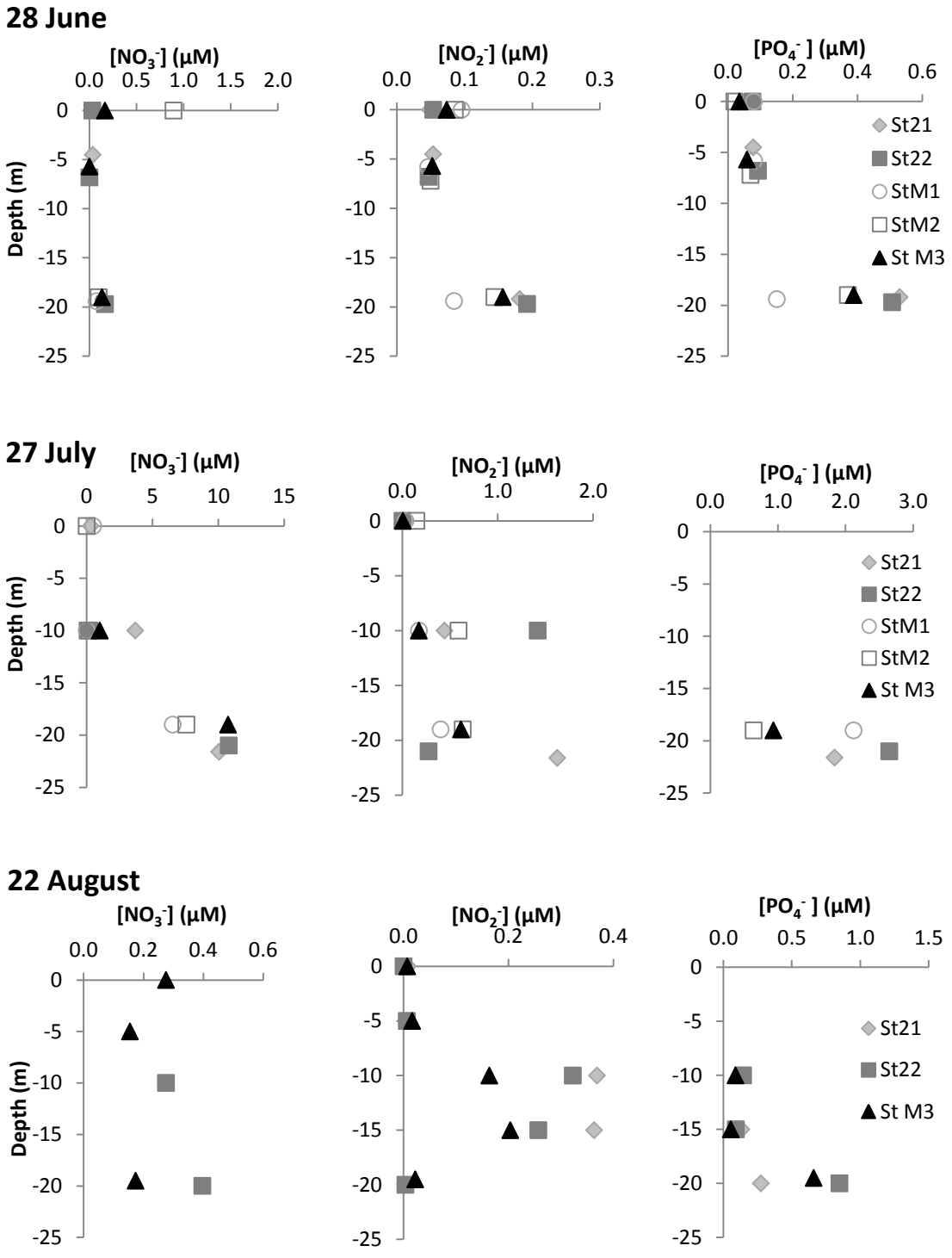


Figure 3-20: Vertical profiles of nitrate, nitrite and phosphate concentration (µM) in June, July and August 2012.

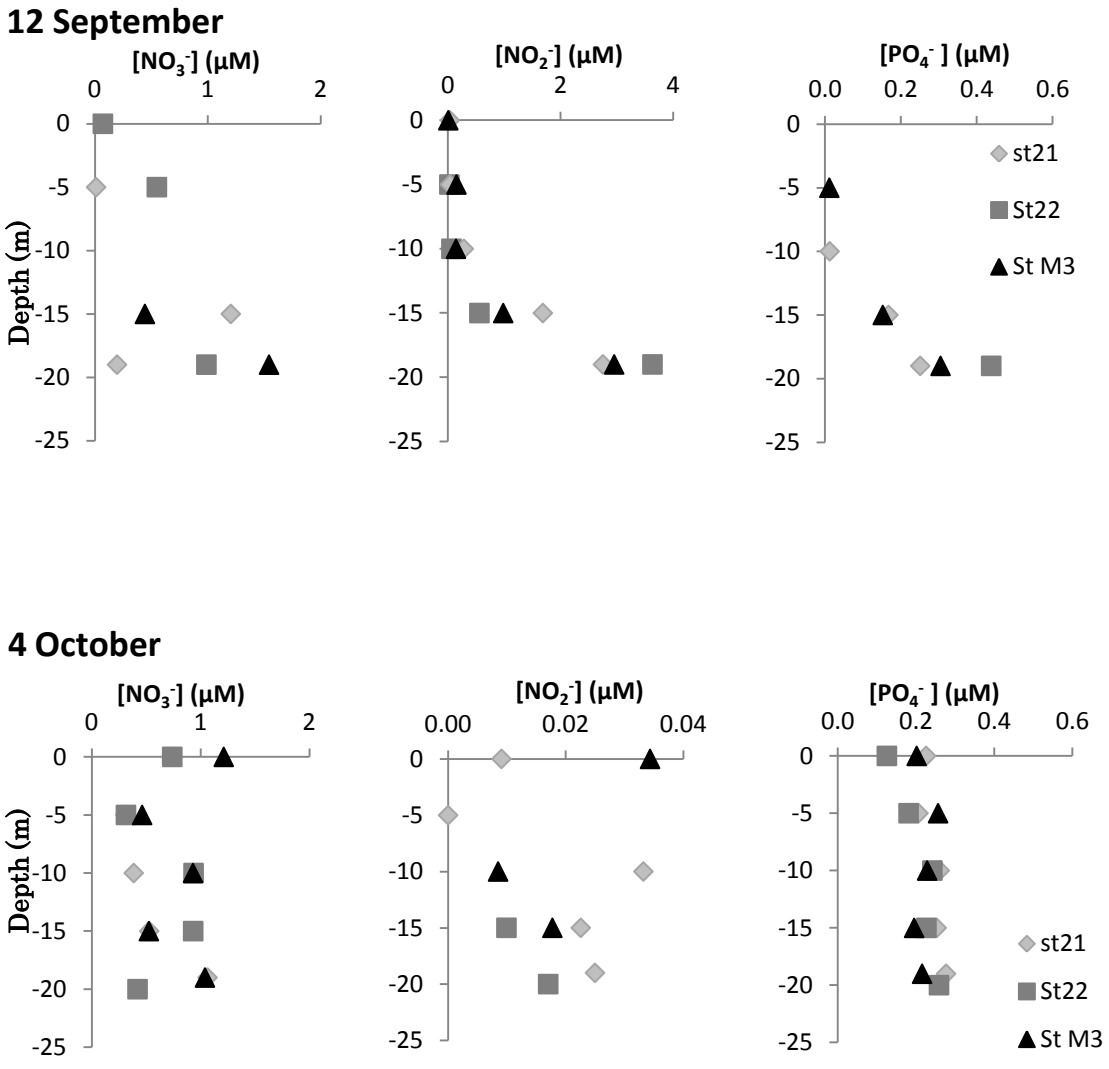


Figure 3-20 continued: Vertical profiles of nitrate, nitrite and phosphate concentration (µM) in September and November 2012.

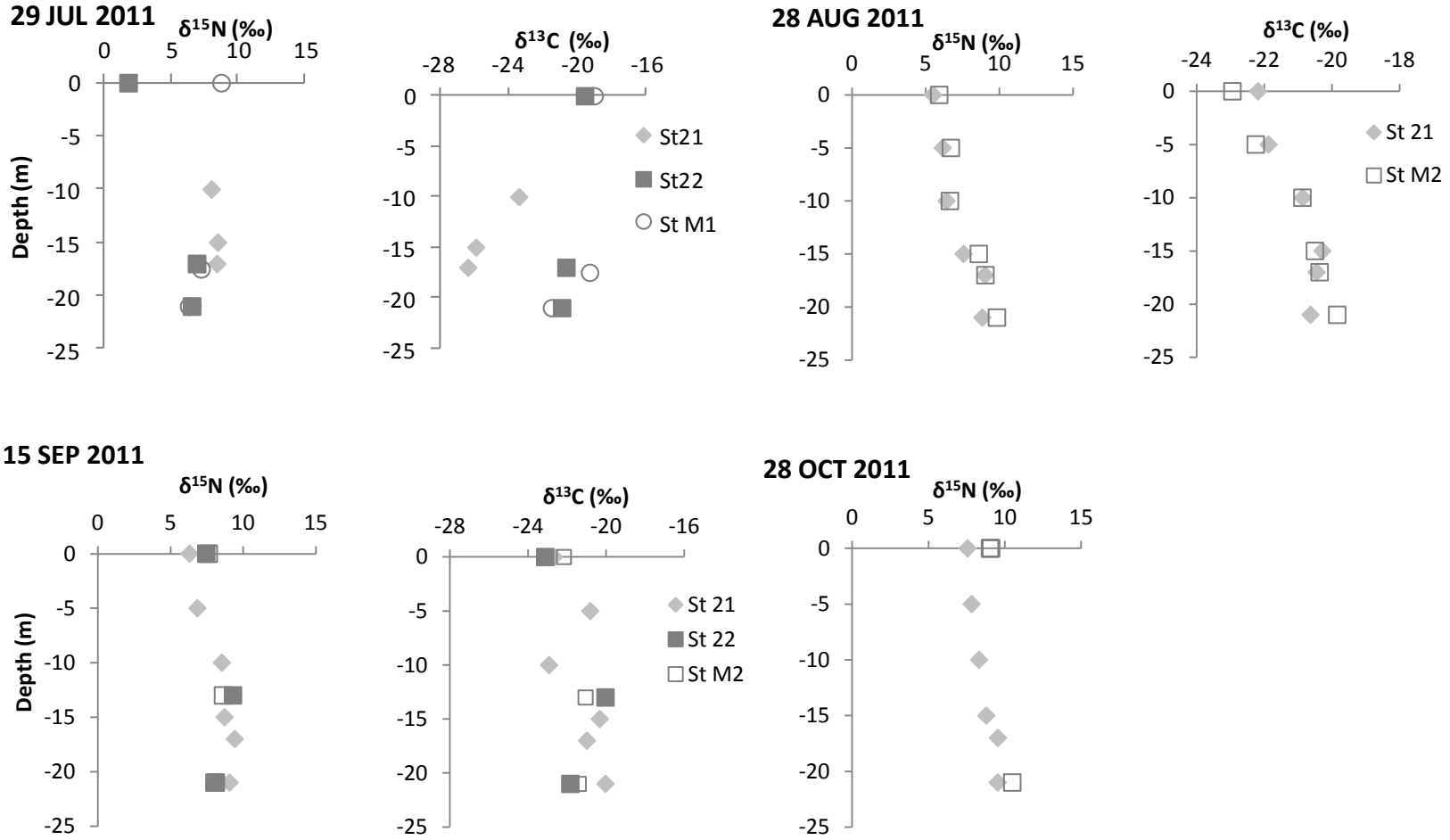


Figure 3-21: Nitrogen and carbon isotopes at Sts. 21, 22, M1 and M2 in the first sampling year of 2011.

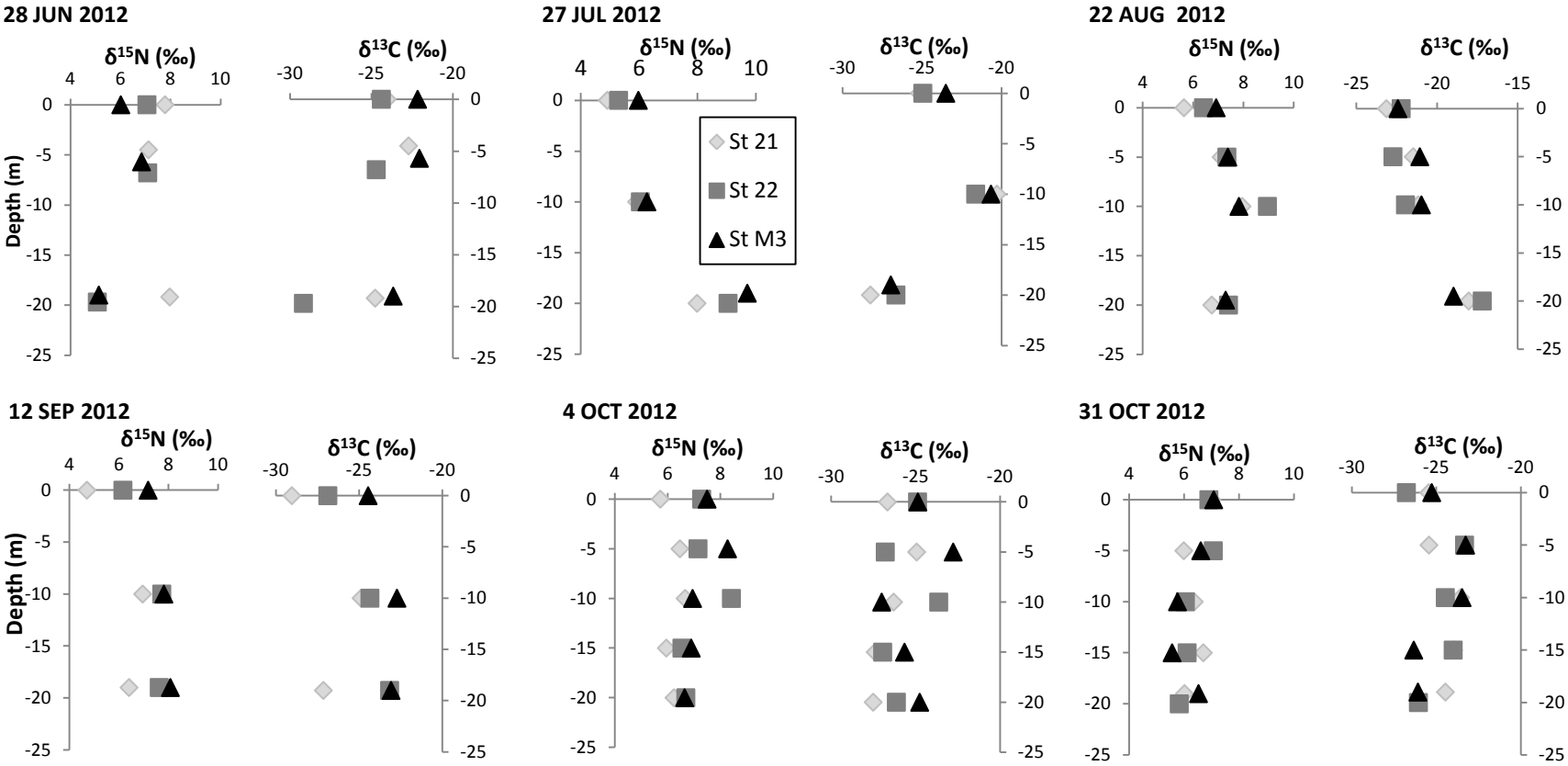


Figure 3-22: Carbon, $\delta^{13}C$ (%), and nitrogen ($\delta^{15}N$, %) isotopes vertical profiles for Sts. 21, 22 and M3 in 2012.

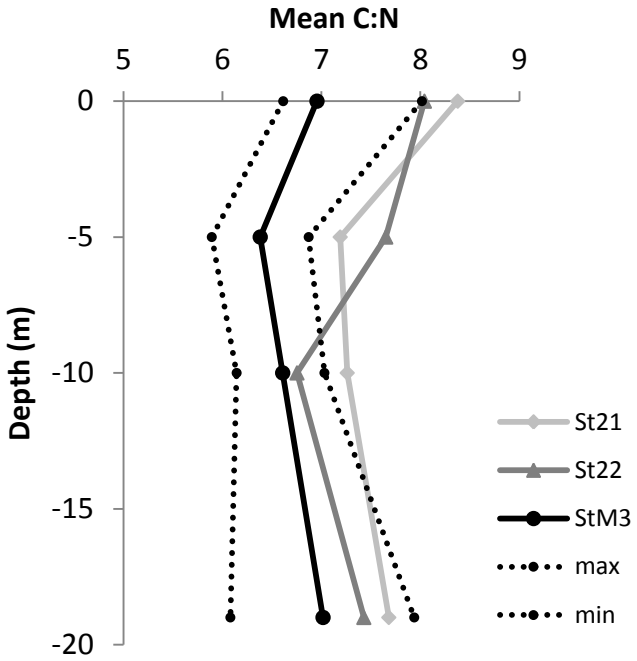


Figure 3-23: Mean values of POM C:N ratios for Sts. 21, St. 22 and St. M3 (solid lines) in 2012. Dashed lines indicate the maximal and minimal values measured at St. M3.

Table 3-1: C:N ratios and C:Chl a ratios in 2012.

Sampling	Station	Depth (m)	C/N	C/ Chl a	Sampling	Station	Depth (m)	C/N	C/ Chl a
28 June 2012	21	0	8.83	74.18	**12 September 2012**	21	0	9.43	64.06
		-4.5	7.49	23.62			-10	7.56	35.29
		-19.2	7.73	130.68			-19	7.82	47.91
	22	0	9.88	46.26		22	0	7.71	75.27
		-6.8	8.03	14.46			-10	6.34	59.80
		-19.7	9.90	93.75			-19	6.48	36.40
	M3	0	6.61	58.37		M3	0	6.86	104.98
		-5.7	6.87	24.95			-10	6.14	28.95
		-19	7.51	54.24			-19	6.54	31.68
	M1	0	6.80	73.62	**4 October 2012**	21	0	9.13	78.31
		-5.8	7.49	18.79			-5	8.49	48.25
		-19.4	9.65	210.35			-10	7.92	55.76
27 July 2012	21	0	6.81	96.40			-15	8.40	53.35
		-10	7.51	44.36			-20	9.58	50.71
		-20	8.66	108.14		22	0	8.98	74.57
	22	0	7.17	99.63			-5	8.51	46.78
		-10	7.21	73.01			-10	8.24	67.05
		-20	7.27	60.33			-15	8.43	55.17
	M3	0	6.33	92.88			-20	7.80	55.58
		-10	7.03	45.79		M3	0	8.93	30.78
		-19	7.94	75.32			-5	7.22	69.31
	M1	0	6.16	163.95			-10	8.66	35.91
		-10	5.52	36.94			-15	8.31	44.88
		-20		0.00			-20	9.14	59.99
22 August 2012	21	0	8.44	167.75	**31 October 2012**	21	0	7.71	38.35
		-5	6.89	92.71			-5	7.30	57.91
		-10	6.72	112.68			-10	6.65	56.18
		-20	6.51	94.18			-15	6.59	55.19
	22	0	7.40	145.15			-19	7.52	106.62
		-5	7.27	116.77		22	0	8.07	85.40
		-10	6.72	95.43			-5	6.96	59.35
		-20	6.06	90.18			-10	6.93	62.68
	M3	0	8.02	131.86			-15	6.46	47.03
		-5	5.89	171.48			-20	8.25	113.60
		-10	6.65	97.14		M3	0	8.34	103.57
		-19.5	6.08	32.13			-5	7.37	71.68
							-10	6.57	52.46
							-15	7.79	102.91
							-19	8.72	113.26

Chapter 4.

Effects of artificial upwelling on the environment and reared oyster *Crassostrea gigas* conditions at Seihi area, Omura Bay

4.1 Introduction

Crassostrea gigas mortalities outbreaks have been reported during summer seasons in many mariculture grounds (Cháves-Villalba, 2007) as related to multi-factors. In summer stratified enclosed bays, the combination of high temperatures and hypoxic conditions seems to make oysters vulnerable to pathogens and cause stress in a period when metabolic demand is usually high and energy reserves are low, due to reproduction. Previous researches about artificial upwelling have described the method as applicable to shellfish aquaculture industry. However, no tests were carried out to verify the hypothesis of improved environmental condition on the performance of bivalves, a critical point in deciding whether or not to install artificial upwelling in bivalve mariculture areas. To ascertain previous researches affirmation, the scientific approaches should assess the condition of oysters submitted to the artificial upwelling effect.

A rapid measure of overall health or ecophysiological state of molluscs is the condition index. The index is regarded as a useful measure to recognize nutritive state of bivalves, to follow gametogenesis activity, besides being an important indication of dissimilarity in the commercial quality of different populations of organisms. Although it has been argued that condition index analysis alone should not be used in ecophysiological studies; it is regarded as a good index of oyster health if used together with collaborating growth and environmental data (Brown & Hartwick, 1988).

Another method to check good condition of shellfishes is by checking the amount of energy reserves in their body. Glycogen is one of the main energy reserves in bivalves and it is regarded as quantitative measure of physiological changes. It is also a measure of health because storage activity usually occurs under favourable environmental conditions and mobilisation and conversion of reserves usually occur during high metabolic demand activity, such as reproduction (Patrick et al., 2006, Li et al., 2010). The physiological state of bivalves should be taken into account with relation to the environmental characteristics in order to address the mortalities occurring in the commercial growing areas (Berthelin et al., 2000).

4.2 Objectives

The aim of this study was to investigate results of the artificial upwelling on possible oyster health improvement, by assessing not only environmental parameters but the connection with those and oyster overall condition.

4.3 Materials and methods

4.3.1 Study area and aeration set up

The Seihi oyster culture area has a mean depth of 6.0 m and open contact with the larger Omura Bay on its western side (Fig. 4-1). Freshwater flows into the bay from a small river, the Daimyoji River located on the eastern side of the Bay. Oysters are farmed in the southern part of the Seihi area.

Positioned on the bottom of the farming area, the aeration system consisted of 2 concentric circles with radii of 10 m and 15 m, respectively. Air was supplied by 40 air cocks connected to 2 air compressors (Hitachi oil free screw compressor) installed on land at a rate of 0.15 m^3 min^{-1}. Aeration periods comprised two summer seasons: 6 September to 15 November 2011 and 26 June to 15 November 2012.

Cohorts of *Crassostrea gigas* spat which consisted of 3 oysters between 50-60 mm in height, 4 oysters between 60-70 mm, 6 oysters between 70-80 mm, 5 oysters between 80-90 mm and 2 oysters between 90-100 mm in 2011, and 56 oysters with shell height of 21.9 ± 2.8 mm (mean ±standard deviation) in 2012, were used to fill each of 18 cages (top diameter of 40 cm, bottom diameter of 45 cm and height of 15 cm) with a mesh size of 5 mm. Oysters were smaller in 2012 because adult oysters were not available for purchase, as a consequence of extreme decreases in oyster seed production after the Tohoku tsunami of the previous year. To assess the aeration effect,

the cages were placed at different distances from the aeration point (St. B = 0 m, Sts. A and C = 30 m, St. D = 60 m, St. E = 120 m and St. F = 180 m) near the surface (2 m and 0.5 m depth in 2011 and 2012, respectively), middle (3 m in both years) and bottom layers (4 m and 5 m depth in 2011 and 2012, respectively; Fig. 4-1). Cage depths differed between the two years, because in the first year of experiment no differences were found in oyster condition in relation to cage depth, a fact that was attributed to the proximity of the cages. In 2012, cages were hung vertically with at a least 2-m difference in depth, in a further attempt to investigate variation between depths.

Samplings and observations were performed at intervals of approximately 30 days, from 28 August to 15 December in 2011 and from 30 June to 6 November in 2012 (Table 4-1). The sampling on 28 August 2011, which was conducted before the aeration had been turned on, constitutes some control data for the environmental characteristics in summer. The sampling dates for both years are specified in Table 4-2.

4.3.2 Environmental data

Temperature, salinity, fluorescence and dissolved oxygen (DO) concentrations were measured using a CTD profiler (Rinko AAQ125, JFE Advantech). Water samples for chlorophyll analysis were collected on a monthly basis at Sts. B, D (included only in 2012) and F. Station D was included in the water sampling in 2012 because unlike St. F, it is not directly influenced by the Daimyoji River. In 2012, DO recorders were installed

at St. B at 0.5, 3 and 5 m depths and at St. F in 3 and 5 m depths, for the duration of the experiments.

Simple stratification indices based on temperature (ΔT) and salinity (ΔS) were calculated for the St. B water column before (August 2011) and after (September 2011) the first-year aeration by the following formula:

$$\Delta T \text{ or } \Delta S_{\text{Month 2011}} = Xs - Xb$$

where Xs is the surface temperature or salinity at 0.2 m depth and Xb is the temperature or salinity at 6 m depth.

To compare temperature and dissolved oxygen concentrations close and far from the aeration system, differences in these parameters between St. B (centre of aeration) and D (just outside the 30 m region affected by aeration; see results Fig. 4-3) were calculated from CTD profiler data for the whole water column and are expressed as vertically averaged values and standard deviations.

Water samples were collected with a Van-Dorn bottle sampler at the respective cage depths for each year. The water was filtered immediately after collection. Chlorophyll *a* (Chl *a*) samples were extracted from particulate organic matter on GF/F filter in the dark for 12 h by 90% acetone, and concentration was measured using a calibrated fluorometer (Trilogy Laboratory Fluorometer). Extracted Chl *a* concentrations and in situ fluorescence showed good correlation ($R^2 > 0.80$), thus fluorescence was calibrated to Chl *a* concentrations (μg L^{-1}). Water samples of 500 mL collected at the same depths as the oyster cages were preserved with formalin (10%) for diatom biomass determination (cells L^{-1}). GF/F filters were also used for obtaining

POM samples for isotopic measurement in 2012, after elimination of inorganic matter with HCl flumes for at least 24 hs. Carbon and nitrogen isotope analyses were performed as described in Chapter 3. Suspended solid concentrations, expressed as annual means per station, were calculated as difference between dried filter weights before and after filtration and the known amount of filtered water. Dissolved inorganic nitrogen (NO_2^- and NO_3^-), phosphate (PO_4^-) and silicate (SiO_2^-) concentrations were measured using an AutoAnalyzer (Bran-Luebbe, TRACSS 2000).

4.3.3 Oyster performance

Oyster samplings were started in the second month of each sampling season, as oyster cages were placed in the field during the first sampling (Table 4-1).

4.3.3.1 Growth and survival

Growth was recorded at interval of approximately 30 days or annually as mean shell height (from umbo to the valve extremity, in mm) of live oysters in the cage. Percentage survival was recorded based on the numbers of live and dead oysters, and expressed in relation to the oysters initially stocked in the previous sampling or in the beginning of the experiment, to obtain 30 days or annual estimates, respectively. Both parameters were calculated for each station and are expressed as mean values with standard deviations.

4.3.3.2 Oyster condition

In each sampling, up to 3 oysters were collected from each cage and both dry shell weight and dry meat weight were measured to calculate the condition index (CI) of the oysters, which is expressed by:

CI = dry soft tissue weight / dry shell weight x 1000.

Oysters were shucked and weighed after drying in an oven at 60 °C to a constant weight (24-72 h). Although it has been argued that condition index analysis alone should not be used in ecophysiological studies, CI is regarded as a good index of oyster health if used together with collaborating growth and environmental data (Brown and Hartwick, 1988). Additionally, CI is a trustworthy tool for analysing growth of oysters because volume and consistency of body tissues may not conform to the increase in the shell (Yonge, 1960).

4.3.3.3 Oyster muscle isotopes

In 2012, the adductor muscles of the same organisms used for CI, were taken, and then powdered dried and homogenised for isotopes analyses. Isotope analyses were performed as described in Chapter 3. Although different tissues may have different

isotopic fractionation values and turnover rates, muscles were chosen due to easy identification.

4.3.3.4 Oyster mantle glycogen

In each sampling during 2012, up to 3 oysters from each cage were dissected and mantle tissues were identified, extracted and weighed. Homogenised mantle tissue samples (0.02 - 0.1 g) were suspended in 30% KOH, and saponified by heating to 100 °C for 2 h. Precipitates were obtained after the addition of EtOH and overnight cold temperature storage. To the centrifuged precipitates, 2% sodium sulphate and 95% EtOH were added and the mixture was left to cool overnight. Samples were again centrifuged and 1N sulphuric-acid was added to the precipitates to finally obtain the final supernatant. Samples were then treated with cold anthrone solution, boiled for 15 min and cooled. Absorbance of the resulting coloured complex, which indicates the concentration of glycogen in the samples, was measured at a wavelength of 620 nm.

Aeration was expected to locally increase available food and change the water temperature. Therefore, mantle tissues were selected for the glycogen analyses due to reported sensitivity of these tissues to chlorophyll levels and reproduction, which is believed to be triggered by high temperature (Li et al., 2009).

4.3.4 Statistical analysis

Data analyses were performed with PASW Statistics software. Variance homogeneity was tested with Levene's test, and the differences in means between stations were tested by One-Way ANOVA. The significance limit was set to 0.05. Relationships between parameters were evaluated by regression models.

4.4 Results

4.4.1 Hydrology and phytoplankton

Before the aeration period, in a control sampling in August 2011, water column stratification was strong, especially within the top one meter of the surface water ($\Delta T_{Aug2011}$ = 6.1 °C and $\Delta S_{Aug2011}$ = -8.15, Fig. 4-2). Sampled stations showed similar characteristics including well defined thermoclines and haloclines. Dissolved oxygen concentration decreased to as low as 2 mg L^{-1} in the bottom layer during this control month. The first sampling after the aeration was begun, in September 2011, showed a relatively less stratified water column ($\Delta T_{Sep2011}$ = 3.1°C and $\Delta S_{Sep2011}$ = -2.9). With the aeration system turned on, the vertical profiles at Sts. A, B and C changed dramatically, and both years showed similar results. Stratification in the water column was weaker within 30 m of the aeration point. Isotherms and DO isopleths were inclined

toward the surface near the aeration, indicating upwelling of water from lower layers to the surface (Fig. 4-3).

Throughout the aeration period, especially in September and October 2011 and July and September 2012, the water column at St. B was less thermally stratified and more homogeneous in DO than those at Sts. D and F, indicating mixing induced by the aeration (Figs. 4-4 and 4-5). The temperature was 0.9 ± 1.3 °C lower at the aeration spot compared to the water column more than 30 m from it. However, DO was 1.7 ± 1.8 mg L^{-1} lower near the centre of aeration due to upwelling of low-oxygen bottom water.

Hypoxic water formed near the bottom layers in August 2011 and July 2012, but the oxygen concentrations remained higher than the defined hypoxic limit of 3 mg L^{-1} in successive months in both years. From the end of September (when the temperature started to decrease), data from DO recorders indicated an increase in DO concentration in the middle and bottom layers (saturation values close to 100%) at St. B (Fig. 4-6). Moreover, temperatures in the middle and bottom layers were similar during this period and ΔDO reached values close to zero at St. B, indicating mixing. Compared with St. B, St. F did not show the same water mixing trend between the middle and bottom layers, instead showing more oxygen in the middle than in the bottom layer (Fig. 4-6).

The first sampling of 2012 (31 June) showed an increased chlorophyll *a* level between 2 and 4 m depth at St. B compared with the other sampled stations (Fig. 4-7). In September 2012, the surface layer at the same station shows chlorophyll *a* concentration values of about 10 µg L^{-1}, whereas the Chl *a* concentrations at Sts. D and

F were lower at least within 3 m depth. On the other hand, after September, chlorophyll *a* vertical profiles showed lower values in the water column at St. B compared with the other two stations. Furthermore, the values at St. B were entirely homogeneous, while Sts. D and F had chlorophyll peaks at different depths. Vertical profiles of chlorophyll *a* in 2011, a year in which aeration was started in September, showed exactly the same trends as for 2012 for during the same months. In September 2012, diatom biomass was increased at St. B by as much as 5 times at the surface and 3 times at the bottom layer, compared with Sts. D and F (Fig. 4-8). The most abundant diatom species were *Chaetoceros spp*., *Skeletonema spp*., *Thalassiosira spp*., and species of Pennate diatoms.

Suspended solids were slightly different among stations with mean values of 0.018 mg mL^{-1} and 0.016 mg mL^{-1} at Sts. B and F, respectively during the aeration period in 2011. In 2012, mean values were 0.048 mg mL^{-1}, 0.047 mg mL^{-1} and 0.044 mg mL^{-1} at Sts. B, D and F, respectively.

4.4.2 Nutrients

In the first sampling year, no differences in nutrient concentration that could have been induced by aeration were found between Sts. B and F. In the second sampling year, in July and September 2012, the nitrate concentration in the surface layer at St. B was markedly higher than at the other two stations (Table 4-1). Nitrite and phosphate concentrations were low during the observation period at all stations. For those

nutrients, St. B showed markedly low values in the July and September samplings, with maximum values occurring in the bottom layer (maximum values at 5 m depth of NO_2^- = 0.35 µM and PO_4^- = 0.41 µM). Dissolved silicate concentrations were similar among Sts. B, D and F at the beginning of the experiment. However, silicate concentrations subsequently decreased in the surface layer at St. B in September and October, whereas St D. and St. F maintained relatively higher values in the same layer (Table 4-1).

4.4.3 Isotopes

Isotopic values of POM varied from -22.47‰ to -27.30‰ in the aeration spot (St. B), and from -20.11‰ to -25.55‰ in the furthest station from aeration point (St. F). They were not significantly different among stations but showed slightly enriched $\delta^{13}C$ values towards the small river (close to station F, mean= -23.4‰) (Fig. 4-9). Oyster muscle isotopes did not show any tendency with mean $\delta^{13}C$ values of -17.48, -17.67, -17.61, -17.71, -17.36, -17.44 for Sts. A, B, C, D, E and F, respectively. Both $\delta^{13}C$ and $\delta^{15}N$ for oyster muscles were more enriched than POM values, with an average increase in 6.43 ± 1.9‰ for carbon isotopes and 2.68 ± 1.7‰ for nitrogen (Fig. 4-10 and Table 4-2). The distinction among the oyster mantle isotopes was not significant, although distinction between oysters and POM samples was clear (Fig. 4-10).

4.4.4 Oyster crop performance

Oyster growth had a final mean of 90.0 ± 1.2 mm in 2011. Growth was similar among all stations and showed no pattern of differences among depths. In 2012, oysters at St. F (71.1 ± 13.8 mm) had the best final growth, followed by those at St. E (68.4 ± 13.0 mm) and St. B (65.3 ± 12.7 mm), although the best growth fluctuated across stations and months. Survival rates in 2011 did not differ among stations or among depths in any month. Final survival was lower (mean = 80.3%) in September compared to the other months. In 2012, final survivals were higher at Sts. D, E and F (mean = 38.9%) than at Sts. A, B and C (mean = 16.7%). At St. F surface and bottom cages oysters survival (92% and 50%, respectively) outperformed the other stations at same layers, followed by St. D (both 50%) and St. B (41.7 and 25%, respectively). Bottom layer cages showed the lower survival rate in all stations. A distinctively high survival rate in this year was achieved by oysters at the St. F at surface layer cage (mean = 91.7%).

Significant negative relatioships were found in 2011 between the distance from aeration and CI of oysters per station ($F= 8.21$, Sig. $F < 0.05$, $R^2 = 0.67$, $p < 0.05$; Fig. 4-11) and per cage ($R^2 = 0.61$, $p < 0.01$). In 2012, the CI was generally higher at stations closer to the aeration point, but the difference was not significant per station ($F = 3.09$, Sig. $F = 0.15$, $R^2 = 0.43$, $p = 0.08$; Fig. 4-11) or per cage ($R^2 = 0.16$, $p = 0.92$). Relationships between CI and environmental parameters were not significant for the entire experimental period. However, in the first month of CI sampling in 2012, CI was

negatively related to temperature and DO ($R^2 = 0.58$, $p < 0.01$ and $R^2 = 0.56$, $p < 0.01$, respectively) and positively related to chlorophyll *a* ($R^2 = 0.37$, $p < 0.05$) in the middle and bottom layers.

Glycogen values were significantly higher in oysters grown in the surface cages in 2012 (ANOVA, $p < 0.05$). However, glycogen levels in oysters did not differ significantly among stations (ANOVA, $p = 0.63$) when all depths were considered. At all stations, the glycogen contents of all oysters decreased sharply in the second sampling (September 2012), but recovered by November, the time of the last sampling. Glycogen trends were followed by CI at all stations (Fig. 4-12). Glycogen showed a negative logarithmic relationship with mortality ($y = -7.64 \ln x + 26.0$; $R^2 = 0.29$, $p < 0.01$; Fig. 4-13a). In contrast, glycogen concentration in mantle was positively related to DO concentration ($R^2 = 0.35$, $p < 0.001$) in 2012 (Fig. 4-13b).

4.5 Discussion

Artificial aeration was employed on the bottom of a semi-enclosed bay in an attempt to boost production in an oyster farm by improving water quality and thereby producing healthier oysters in summer. The collected data consisted of hydrological parameters and biological measurements of oysters at different distances from the aeration point. As will be discussed, the aeration was shown to change the environmental conditions in the water column, especially at the beginning and end of the summer season. Because the experiment was started in the middle of summer during

the first year (August 2011), it was considered that data could not show positive effects which may have resulted during that year, had the experiment started earlier. As a result, 2012 data provide a more accurate demonstration of the potential outcomes of the aeration during the summer season.

The general effects of artificial upwelling were investigated by comparing the periods of aeration with a control sampling collected when the aeration was off (August 2011), as well as with comparisons among stations located at progressive distances from the aeration point. Most of the data showed no differences among depths, therefore these comparisons will only be discussed when relevant. Specific effects of the aeration are discussed based on the results of samplings prior to oyster spawning season.

From the CTD data it was possible to establish two distinct areas: an area within 30 m of the aeration, where the effects of bubbling could be detected and the water column was mixed, and an area farther from the aeration, where the water column was stratified and patterns of temperature, salinity, and oxygen stratification were similar (Fig. 4-3). The temperature in the former area decreased because of the constant upwelling of cold bottom water towards the surface. Surprisingly, the aeration did not increase DO concentration in the water near the aeration point. Instead, cold, nutrient-rich but low-oxygen water reached the surface. This is at odds with our expectation of improved DO concentration, and it shows that factors such as gas dissolving rate and instantaneous water characteristics have to be considered in order to set the appropriate aeration rate to increase the DO concentration in the water (Wüest et al., 1992; Strand, 1996).

In the study area, hypoxic water formed in the summer of both years of the experiment. Water with low DO concentration was detected in early summer samplings, during August 2011 and July 2012 (Figs. 4-4 and 4-5). The former sampling occurred prior to the beginning of aeration, but aeration was being performed during the latter, and was not sufficient to counter the formation of hypoxia. Rather than being affected by the aeration system, the formation of oxygen-deficient water in the bottom layer from July to September was a result of seasonal variation. Above the 4 m depth and within 30 m of the aeration point, the aeration reduced DO concentration due to upwelling of low-oxygen waters previously concentrated at the bottom. Fortunately, levels of oxygen in the water column were sufficiently high (> 3 mg L^{-1}) to accommodate this change. Thus, the negative impact of lower oxygen on the oysters is believed to have been secondary to the effects of high temperature. The aeration increased DO concentration only at the end of summer when temperature was already mild, maintaining mixed middle and bottom layers (Fig. 4-6).

No effect of aeration on suspended solids was detectable in the data. Nevertheless, the concentration of suspended solids at the aeration point (St. B) was similar to that at St. F, which was close to the river.

The pattern of nutrients differed between the water column near the aeration and that at more distant stations (Table 4-1), and this was related to primary production. Phytoplankton, whose production is often limited by nitrogen and/or phosphate, almost immediately take in these nutrients that were supplied at higher than normal rates from upwelled water (Sakshaug and Olsen, 1986). In June 2012 the water column at St. B between 2 and 4 m depths increased Chl *a* concentration and in July nutrients showed

higher values at the surface at St. B than at the other stations. With abundant nitrogen, the quick utilisation of phosphate in the food-web might explain why it was not possible to detect higher concentrations of phosphate close to the artificial upwelling in the data. In September, nutrient-rich water reached the surface enabling a phytoplankton bloom. This bloom seems to have consumed the nutrients and, together with the on-going mixing of the water column that caused nutrient dilution, prevented the support of late summer primary production in October and November at St. B (Figs. 4-7 and 4-8). On the other hand, Sts. D and F showed higher primary productivity in late summer, as nutrients from the bottom layers were redistributed to upper layers due to decreased water column stratification.

At St. B it is possible that nutrient dilution was one of the outcomes of artificial upwelling, as already described in models by Williamson et al. (2009). These researches called attention to the importance of determining the minimum nutrient concentration required to sustain a viable phytoplankton population in the area intended to host an artificial upwelling. Nonetheless, St. B maintained higher nutrient concentrations during the beginning and end of each sampling year, suggesting that the aeration promoted increased nutrient concentrations were promoted by the aeration. Phytoplankton abundance at St. B was also higher than the other control stations, possibly due to upwelling of nutrients and cysts of microphytobenthos in the sediment (e.g., Fuji and Matsuoka, 2006).

Dominance of diatoms over dinoflagellates in the phytoplankton community represents a desired situation in relation to food quality for bivalves, because diatoms are higher quality food than are dinoflagellates for bivalves (Strand, 1996). Previous

work indicated that both dinoflagellates and diatoms are present in Omura Bay (Fuji and Matsuoka, 2006; Ishii et al., 2011). Species of the former dominate in oligotrophic stratified water columns, whereas the latter often dominate in weakly stratified and homogeneous waters (Cushing, 1989). The three most abundant diatom species found in Seihi, *Chaetoceros spp*., *Skeletonema spp*., and *Thalassiosira spp*, are well known for forming resting spores (Ishii et al., 2011; Wang et al., 2013). Increases in diatom biomass at Seihi are believed to be a result of nutrient availability and resting stage cells suspended in the water column due to the aeration.

Particulate organic carbon isotopic (POC) values differed from the normal estuarine tendency of enriching gradient from freshwater run out (~ - 25‰, if due to terrestrial plants) towards higher salinity areas (~ - 21‰, if due to marine phytoplankton), showing the most negative values at station B at least at surface. It was expected a broader isotopic values among stations, in which would be possible to identify heavy $\delta^{13}C$ isotopes due to ressuspended microphytobentos (~ - 16‰) near to the aeration system, but this did not occur. However, the middle and bottom layers of station B had always similar values to the same layers at station F, usually lighter than station D. If in both stations the water column in those depths shared same characteristics and had similar turbulence, in the first due the aeration, in the latter due to the river, POM isotopes would reflect similar patterns. Moreover, benthic microalgae is ressuspended together with a considerable amount of detritus, which is also placed in suspension and which are not accounted for in the POM filter weights, and would then lighten the water column POC to values in between the heavy ones from microphytobenthos and estuarine sediments (Riera & Richard, 1996).

$\delta^{15}N$ values in oysters varied from 9‰ (St. A, B) to 8.6‰ (St. E), typical values for marine waters. Significant differences among carbon and nitrogen oyster isotopes from different stations were not found. One of the possible reasons is the turnover rate of oyster muscle tissue is slow, and when compared to other tissues was found to be isotopically static both temporally and spatially (Piola *et al.*, 2006). Expectedly, oyster isotopic values were more enriched than POM isotopes, but calculated differences between oyster isotopes and POM surpassed the usual 1‰ enrichment value in filter feeders for carbon isotopes (Table 4-2). Oysters are capable of selecting particles before ingesting, selecting food by size, quality and quantity (Cháves-Villalba *et al.*, 2007). This may lead to high isotopic values in oysters due to selection of isotopically enriched particles, like observed in our data.

Although the final growth of oysters at St. B was relatively good, the shell height at St. F, especially at the surface layer, surpassed that at the other locations. It is usual for oysters grown in surface layers to display higher growth due to abundant food, but at our site, the difference also seemed to be related with the proximity of freshwater. Many bivalves-fouling organisms are suspension feeders and might compete with oysters for food (Gosling, 2004). Freshwater runoff usually kills fouling organisms, favouring oyster growth (Oczkowski et al., 2011; Pollack et al., 2011).

It is known that care is required when measuring growth by shell height because the volume and consistency of soft body tissues may not conform to increases in shell height (Yonge, 1960). Therefore, in addition to shell growth, condition index measurements were obtained because they also take body tissues into account.

Some research has related CI with temperature, salinity, chlorophyll *a* (Rheault and Rice, 1996; Yildiz et al., 2011), dissolved oxygen concentration and sexual maturation (Maldonado-Amparo, 1998; Li et al., 2010; Liu et al., 2010). However, other studies found no direct relationships between bivalve CI and these environmental parameters (Li et al., 2009) or reproduction (Cháves-Villalba et al., 2007). At Seihi, negative relationships of CI with temperature and DO could be detected in the first sampling in the middle and bottom layers, as well as a positive relationship between CI and Chl *a* level. Because DO concentration near the aeration system was lower until the end of the summer season and the results of the present study showed a positive relationship between proximity to the aeration point and oyster CI, oxygen does not seem to have directly affected CI. Despite the DO concentration, by lowering temperature and increasing plankton quality (favouring diatoms) and biomass, the aeration improved water quality and this was reflected in the high CI of oysters in the first samplings. Nevertheless, relationships between CI and environmental parameters were not significant if the entire duration of the aeration was considered, possibly because of the strong negative effects of temperature. When temperature varies in the range of 23°-25°C, just above the optimum for this species (Le Gall and Raillard, 1988; Bourlés et al., 2009), any small difference in temperature may affect oyster condition differently. On the other hand, it is likely that at extremely high temperatures of more than 28 °C, as were observed in August - September, small variations in temperature do not result in different biological responses, implying that the relationship between CI and temperature is non-linear when a wide range of temperatures is considered.

C. gigas mortalities appear to be linked to a sequence of situations. The sequence begins with food availability leading to fast growth, followed in summer by stress induced by oxygen depletion and by high temperature which triggers reproductive activity. In the end, oysters exposed to stressful conditions have low glycogen reserves and are vulnerable to pathogens (Goulletquer et al., 1998). In the 2012 experiment, oysters at Sts. A and C had lower survival rates than those at stations farther from the aeration point, although St. B had a slightly higher survival than those stations and St. E. The synergetic effects of lower oxygen concentration and the season-induced high temperature may have resulted in higher mortality among the oysters during the period of maximum temperature in the summer. Moreover, increased temperature is known to favour pathogen growth at high salinity sites (Kanno et al., 1965; Powell et al., 1994). Indeed, salinity may be the differentiating factor between the mortality rates at the stations close to the aeration point (Sts. A, B and C) and those far from it (Sts. D, E and F). It is well known that freshwater from rivers kills epibionts; thus oysters that are often bathed by low-salinity waters have fewer predators than those at sites where there is almost no direct freshwater input (Oczkowski et al., 2011; Pollack et al., 2011). Unfortunately, epibiont data were not assessed in this work to reinforce the observations mentioned earlier, but a difference in epibionts between stations closer to the freshwater discharge and stations far from it was clear during the samplings periods.

Mortality has also been linked with low glycogen storage (Goulletquer et al., 1998; Li et al., 2010). Glycogen contents in mantle tissues were measured as a second attempt to check the health of oysters. Although glycogen levels were high in July, they dropped considerably at all stations in September (Fig. 4-12). The decrease in glycogen

content in the mantle tissues was followed by decreased CI in the oysters but with a time-lag. Because the biochemical variations in oysters grown in different locations were similar, it seems likely that decreases in mantle-tissue glycogen occurred during a gonadal development. This conclusion is supported by a reproduction study by Li et al. (2010) which described oyster metabolic changes associated with reproduction. Spawning and depletion of glycogen reserves in oysters usually occur after periods of high temperature (> 28 °C), such as the temperatures in September in this work. When gametogenesis begins, the glycogen stored in tissues is used to support gonad development, a developmental period of high metabolic demand (Liu et al., 2010). This increases the CI index of oysters, because energy is allocated from mantle tissues to the development of gonads, which would weigh more during a reproductive stage than the mantle previously did. Chávez-Villalba et al. (2007) reported that high oyster CI during periods of accelerated reproductive activity was associated with mortality events. However in our data, spawning possibly occurred following a small increase in CI. Then, CI started to decline and recovered only after the spawning season, during increasing energy storage, as indicated by the glycogen content. Although the temperature had already dropped by October, glycogen contents remained low, indicating that the oysters were still in a recovery period. Li et al. (2009) showed that oysters needed a month to recover CI levels after spawning, and to concomitantly increase their energy reserve.

As in Li et al. (2009), oyster CI recovery in Seihi seemed to last until November, when oyster glycogen levels rose again. A spawning season from July to September and subsequent decreases in glycogen and CI index have been identified for

Pacific oysters in Japanese waters by Akashige and Fushimi (1992) and were confirmed by our findings.

Glycogen content in the mantle was significantly related to the mortality that occurred between the July and September samplings (Fig. 4-13a). It is known that oysters after spawning are more vulnerable to environmental stress and diseases (Li et al., 2010). Because glycogen was related to DO concentration in the environment through a highly significant relationship (Fig. 4-13b), this suggests another possible cause for differences in mortalities in stations close to and those far from the aeration system.

Glycogen synthesis in *Ostrea edulis* is inhibited by anoxia (DO ~ 0 mg L^{-1}), although inhibition does not take place at low oxygen concentrations (L-Fando et al., 1972). As different species of oysters would be expected to react differently to DO levels and glycogen synthesis inhibition, *C. gigas* may store more energy in tissues with at increased DO levels. Considering both, the oxygen concentration and mortality relationships with glycogen reserves, it is possible to conclude that providing aeration maintains lower water temperature and good levels of dissolved oxygen, oysters could store more energy and increase survival during subsequent spawning periods.

4.6 Conclusions

In summary, this study documented hydrological improvements promoted by artificial upwelling that benefitted Pacific oyster health. The upwelling of cold and nutrient rich bottom waters drastically reduced hydrographical stratification within 30 m of the aeration point. Improvements in water quality included relatively lower temperature, redistribution of nutrients and re-suspension of dormant phytoplanktonic cells leading to increased phytoplankton biomass, and possible phytoplankton quality improvement. It seems however that oysters were actively selecting their food. Although the aeration seemed to have a positive effect on oyster condition (CI), which could be explored with respect to aquaculture logistics, this relationship should be investigated further. At the rate performed, aeration could not overcome negative oxygen and temperature effects on oysters from July to September. As a result, reduced stored energy and CI during reproduction season left oysters vulnerable and mortality followed. However, water quality improved at the beginning of autumn, with optimum temperatures and increased DO levels due to aeration. A further need for an upwelling system is to guarantee the positive benefits on oyster cultures (lower temperatures, improved food and increased oxygen concentrations) throughout the summer season. It is clear that different aeration rates and designs may lead to better results (Williamson et al., 2009; Fan et al., 2013). The data also suggest that at Seihi, oyster cultures would have better results if a river proximal site were used in combination with artificial aeration.

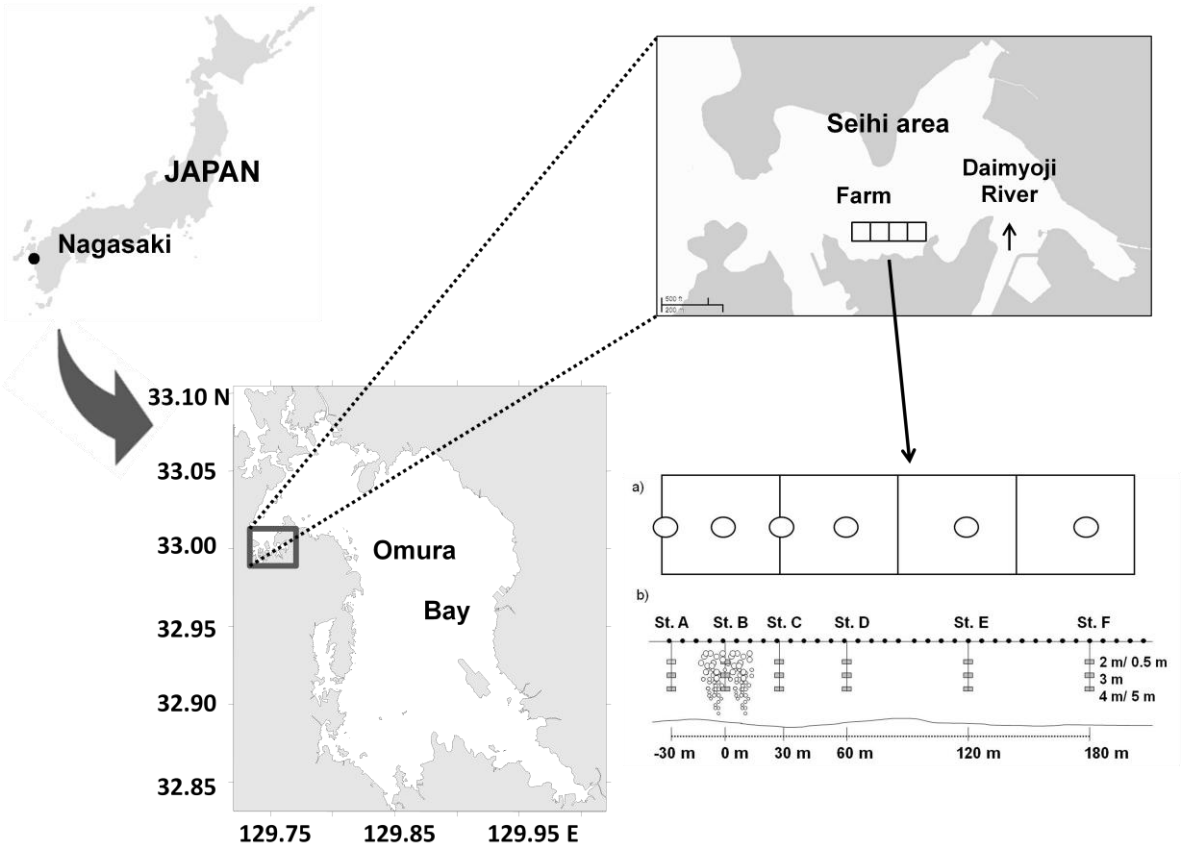


Figure 4-1: Location of Seihi area in the northwestern part of Omura Bay, Nagasaki, Japan and the design of an aeration system: **a)** aerial view, and **b)** vertical view, showing the aeration point at St. B, location of other stations, as well as oyster cage depths in 2011 and 2012.

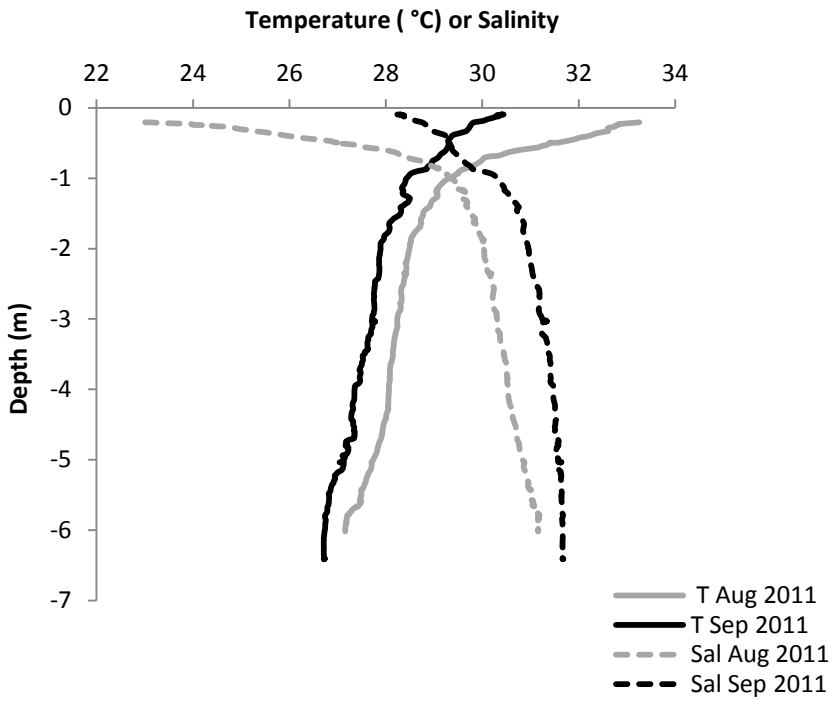


Figure 4-2: Stratification comparison before (31 August 2011) and after artificial upwelling (14 September 2011) at St. B. Full lines represent temperature profiles and dashed lines represent salinity profiles.

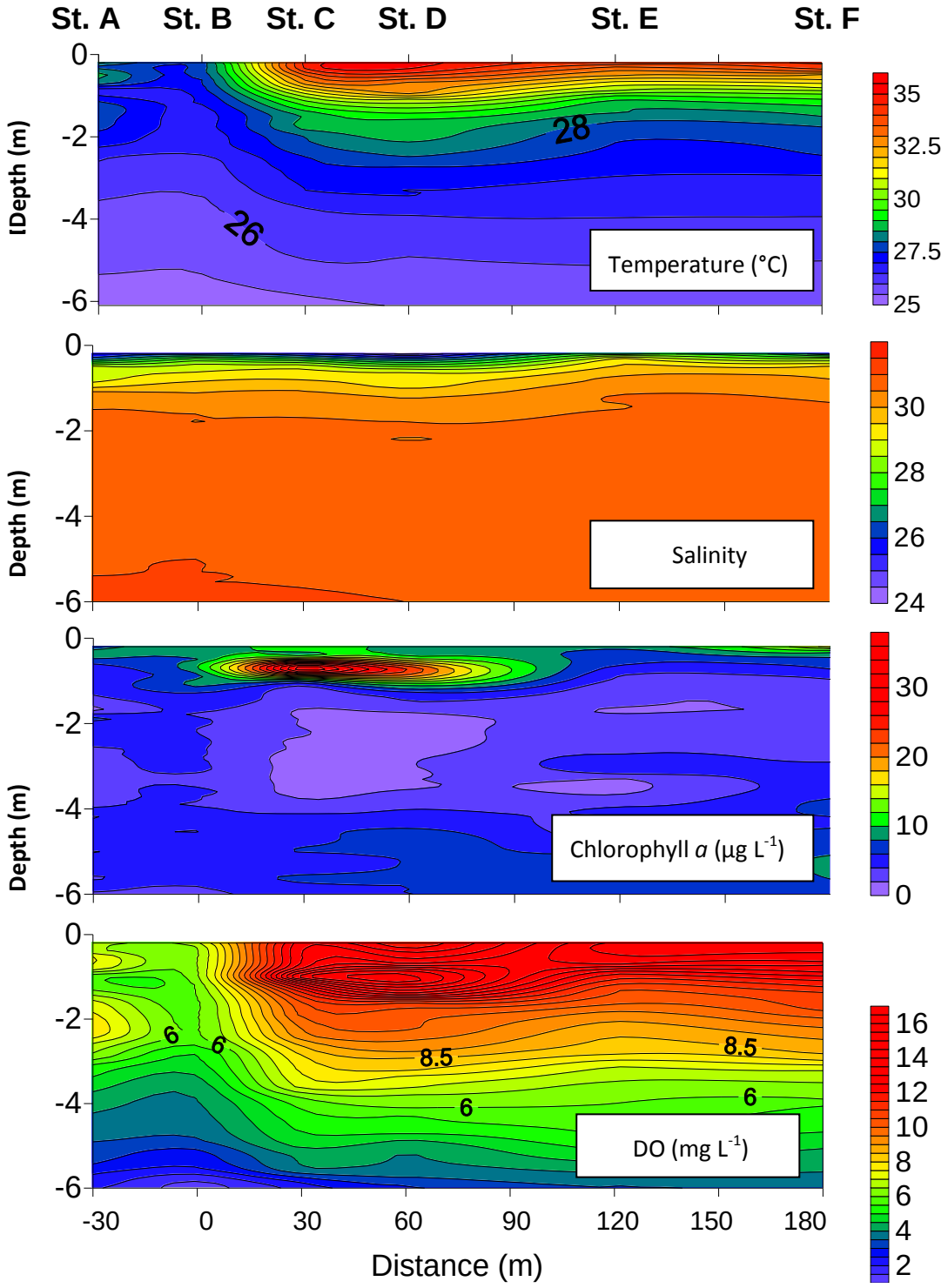


Figure 4-3: Vertical sections of temperature, salinity, chlorophyll *a* and dissolved oxygen on 31 July 2012, after 35 days of aeration.

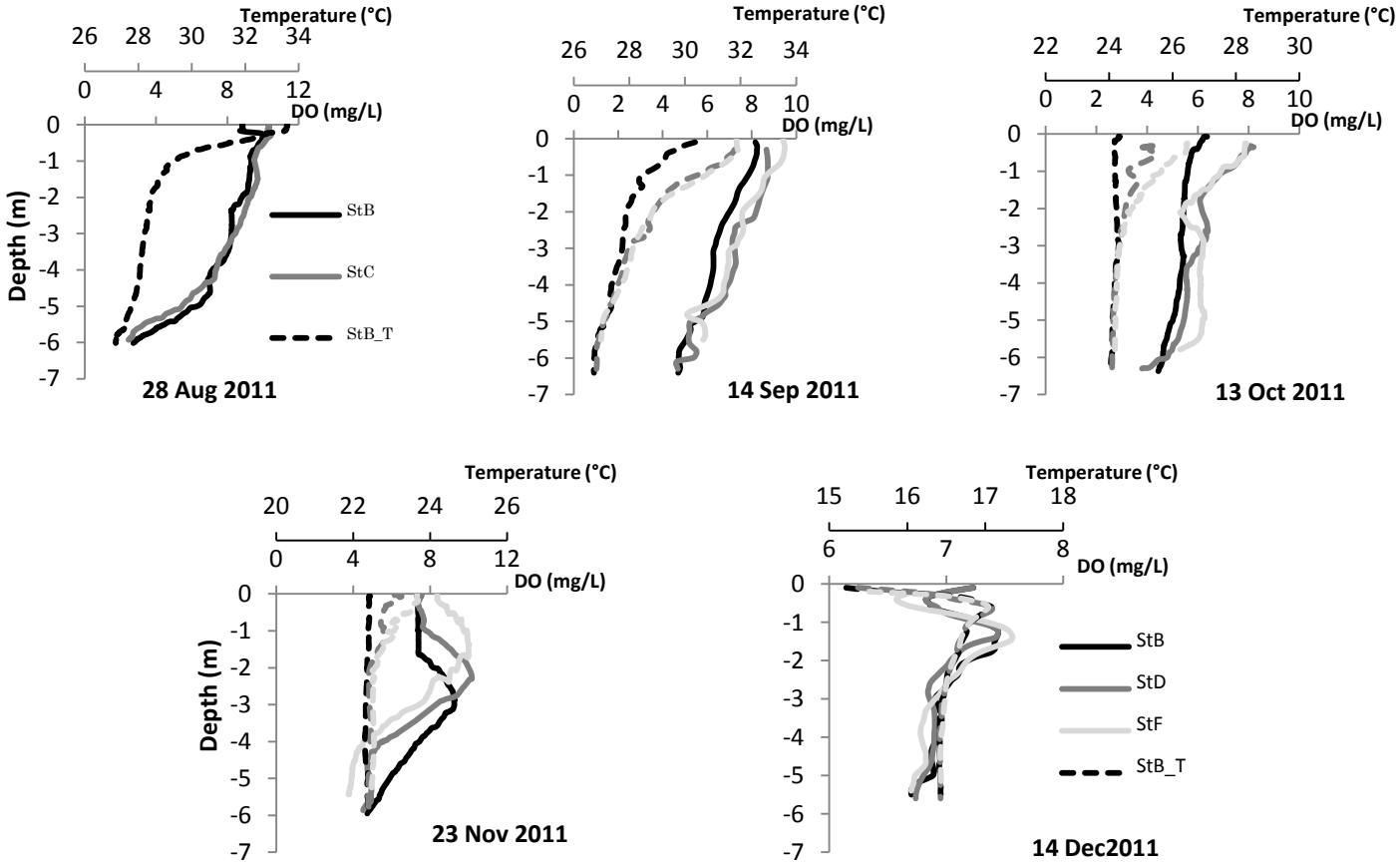


Figure 4-4: Vertical profiles of dissolved oxygen (mg L^{-1}, solid lines) and temperature (° C, dashed lines) at Sts. B, C (in August), D and F in 2011. The legend for August sampling is beside its graph, while other months share the same legend located beside December sampling graph.

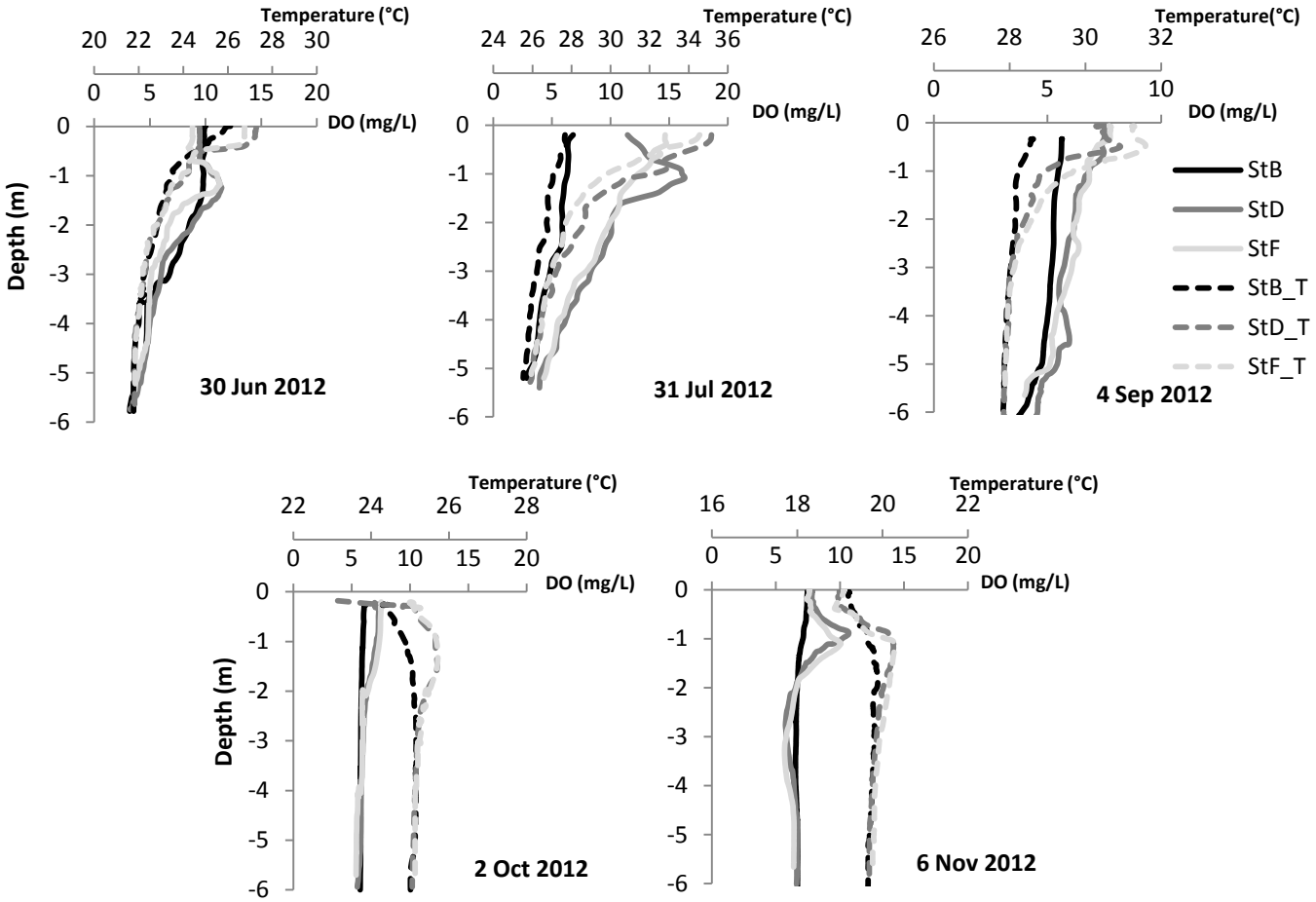


Figure 4-5: Vertical profiles of dissolved oxygen (mg L^{-1}, solid lines) and temperature (° C, dashed lines) at Sts. B, D and F in 2012.

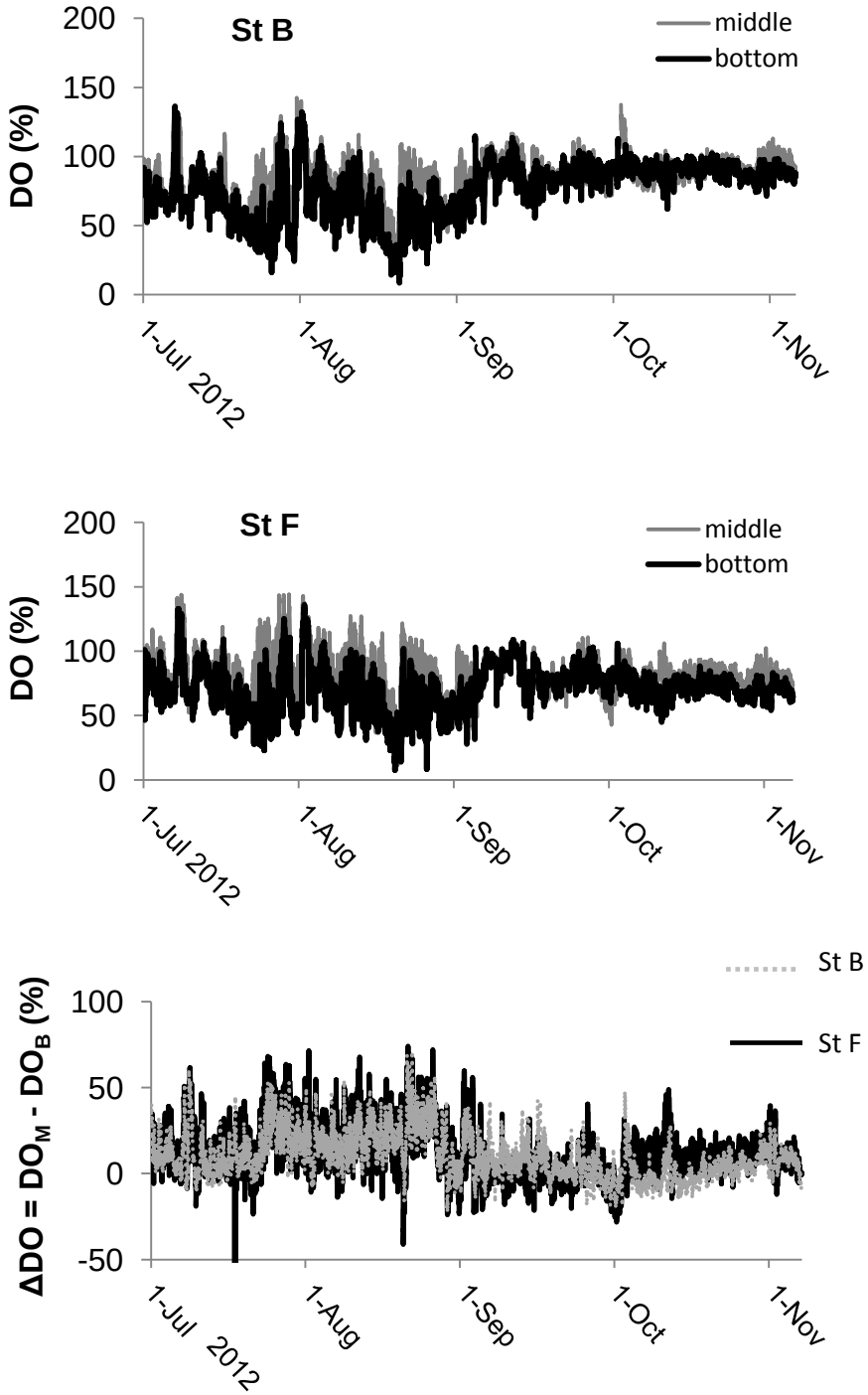


Figure 4-6: Time change in dissolved oxygen (%) measured by installed sensors at Sts. B and F.

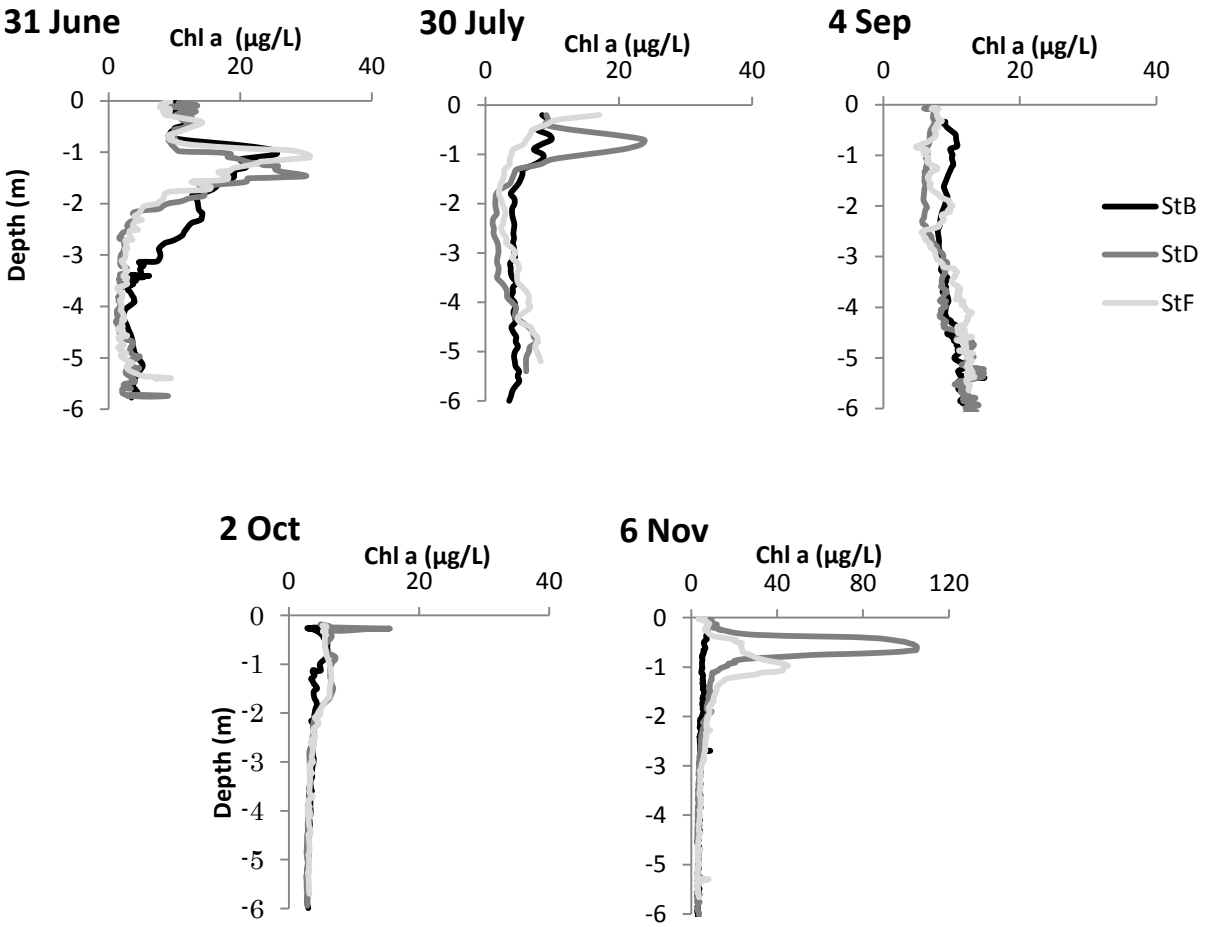


Figure 4-7: Vertical profiles of chlorophyll (µg L^{-1}) at Sts. B, D and F in 2012.

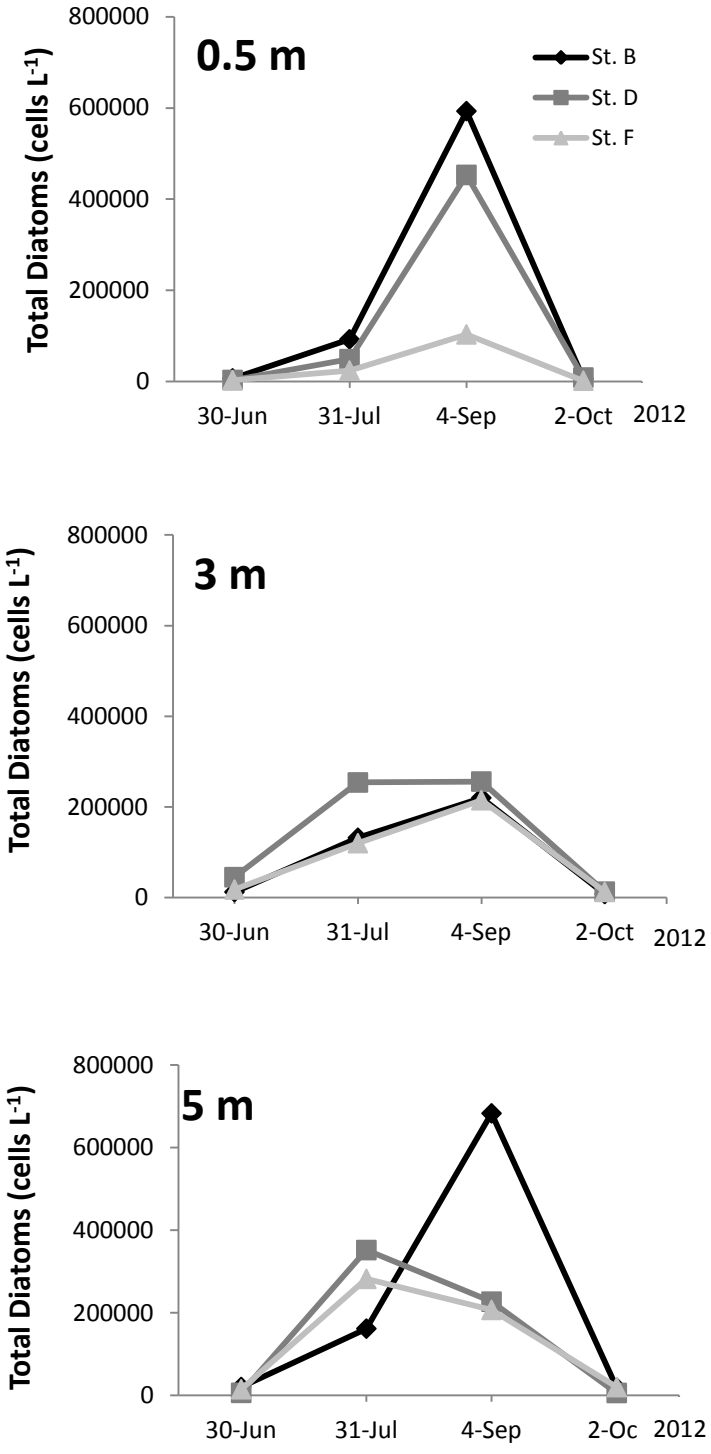


Figure 4-8: Diatom biomass (cell L^{-1}) in 3 different layers at Sts. B, D and F in four samplings in 2012.

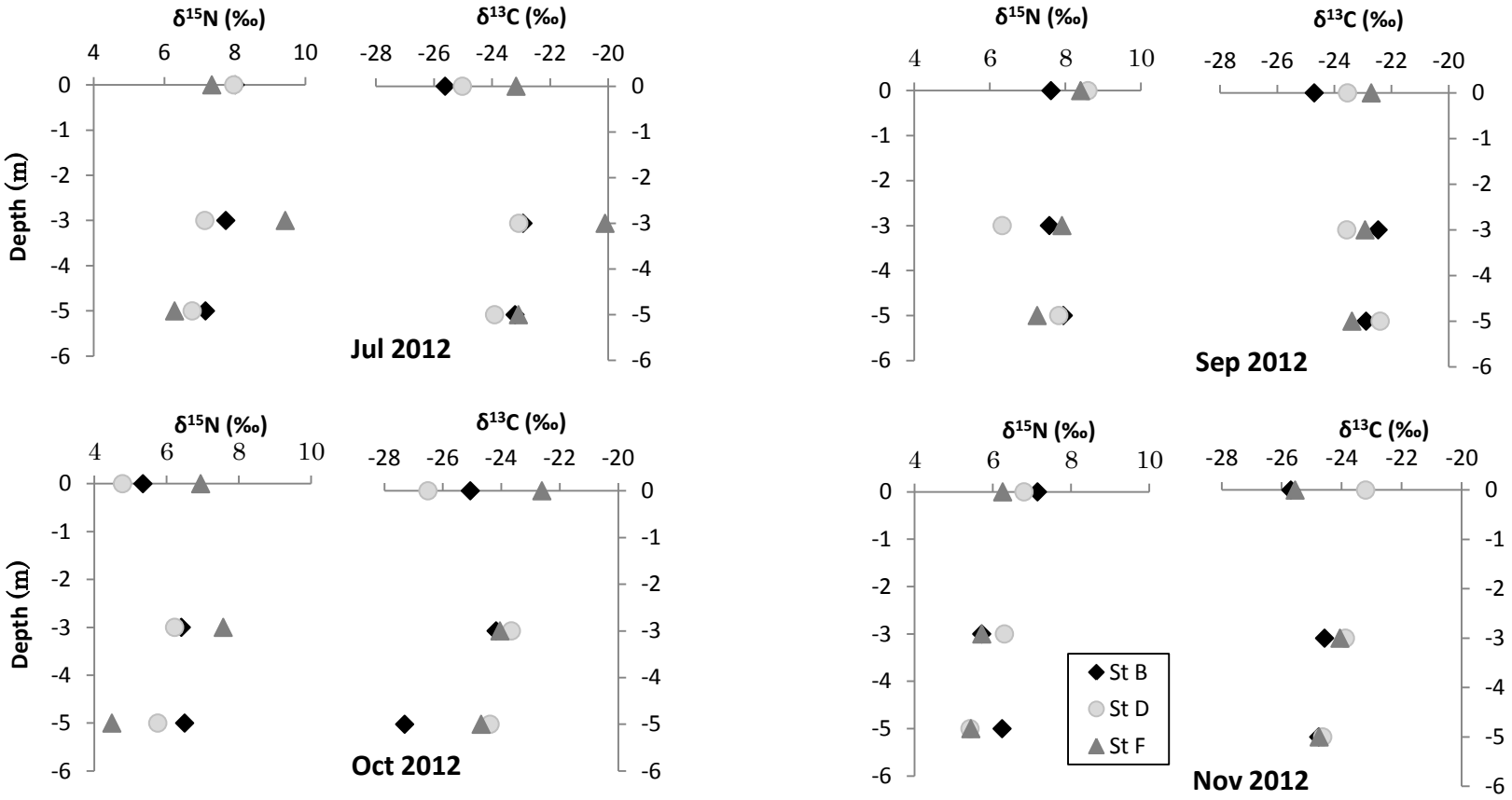


Figure 4-9: POM isotopes collected during sampling of 2012, at Sts. B, D, and F.

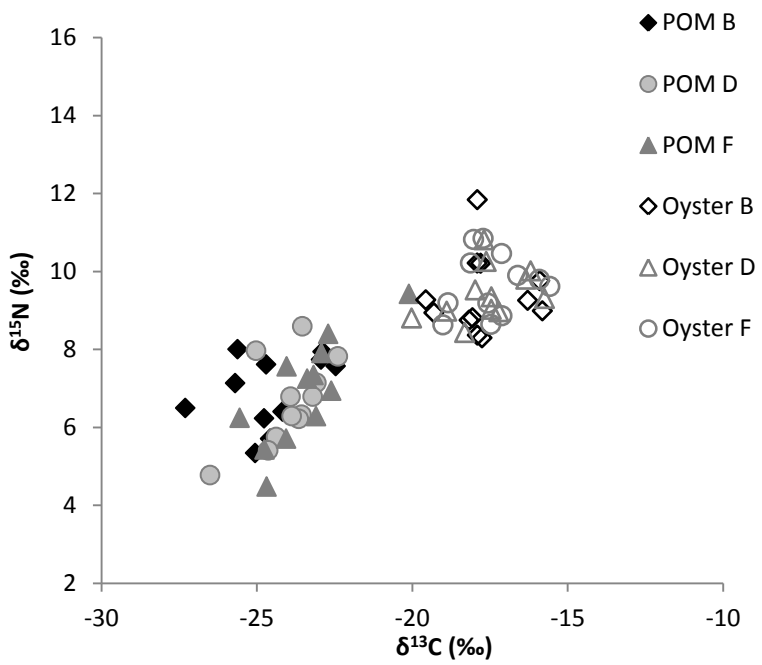


Figure 4-10: Isotopic relationship of POM and oyster muscles.

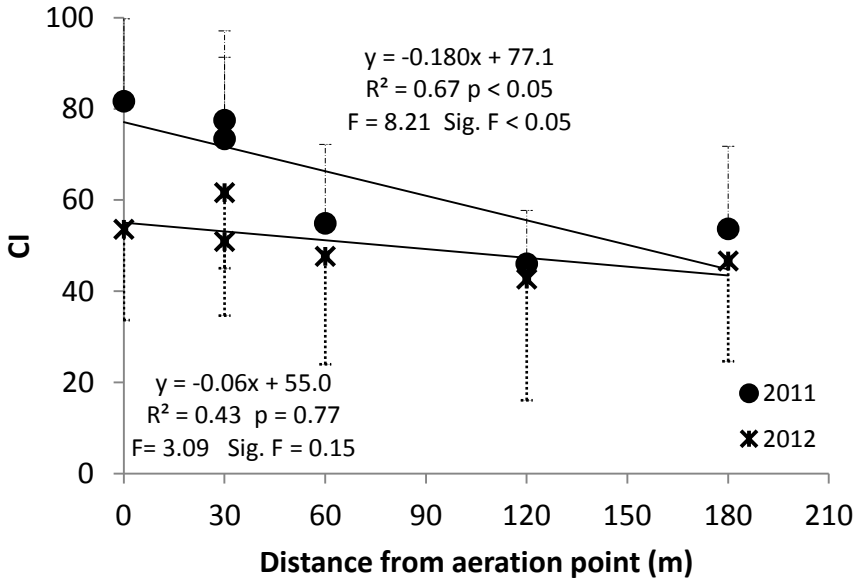


Figure 4-11: Condition index of oysters in relation to the distance from the aeration point in 2011 and 2012.

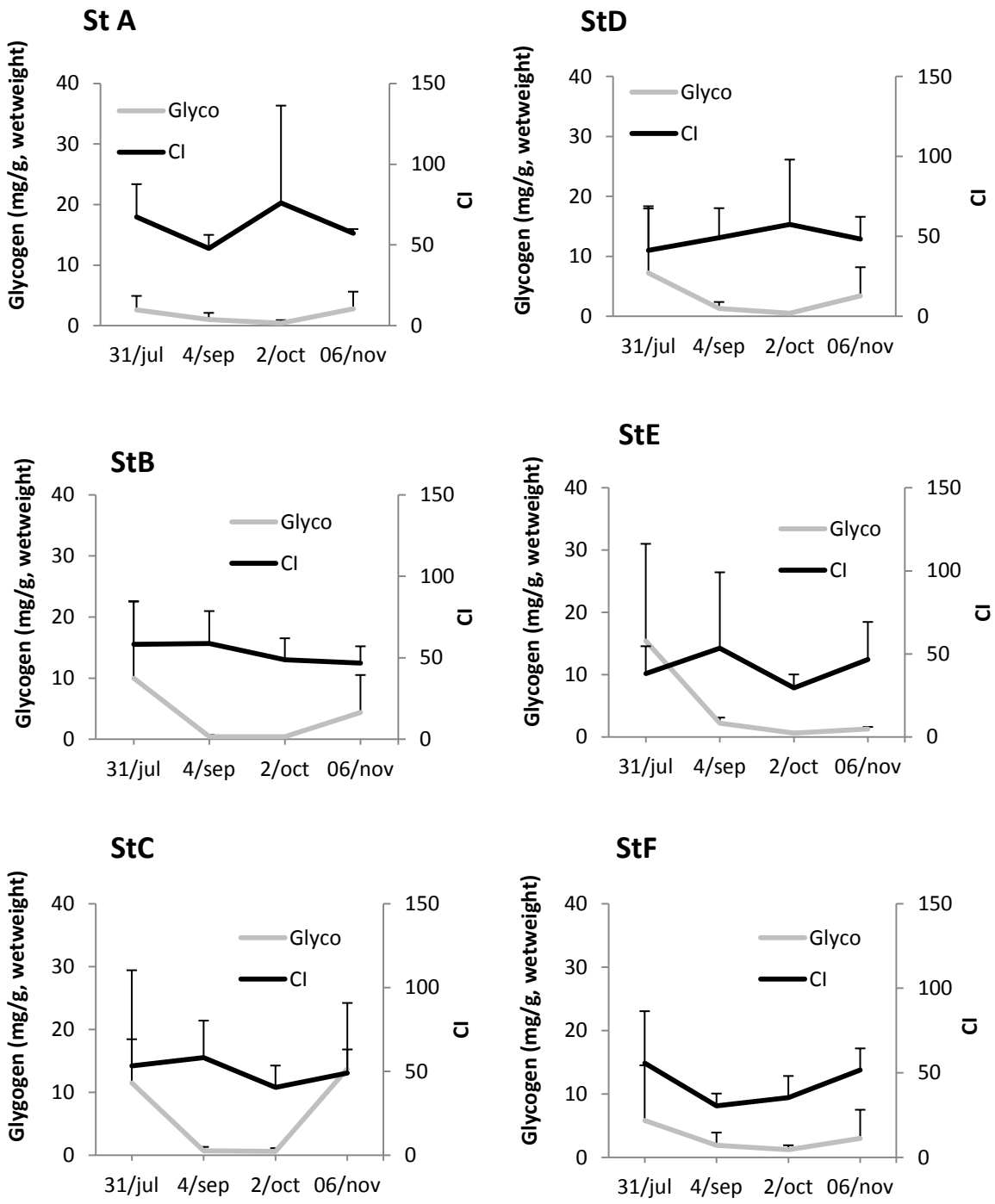


Figure 4-12: Variations in glycogen contents (mg g^{-1}, wet weight) in oyster mantle tissue and CI, through the aeration period in 2012.

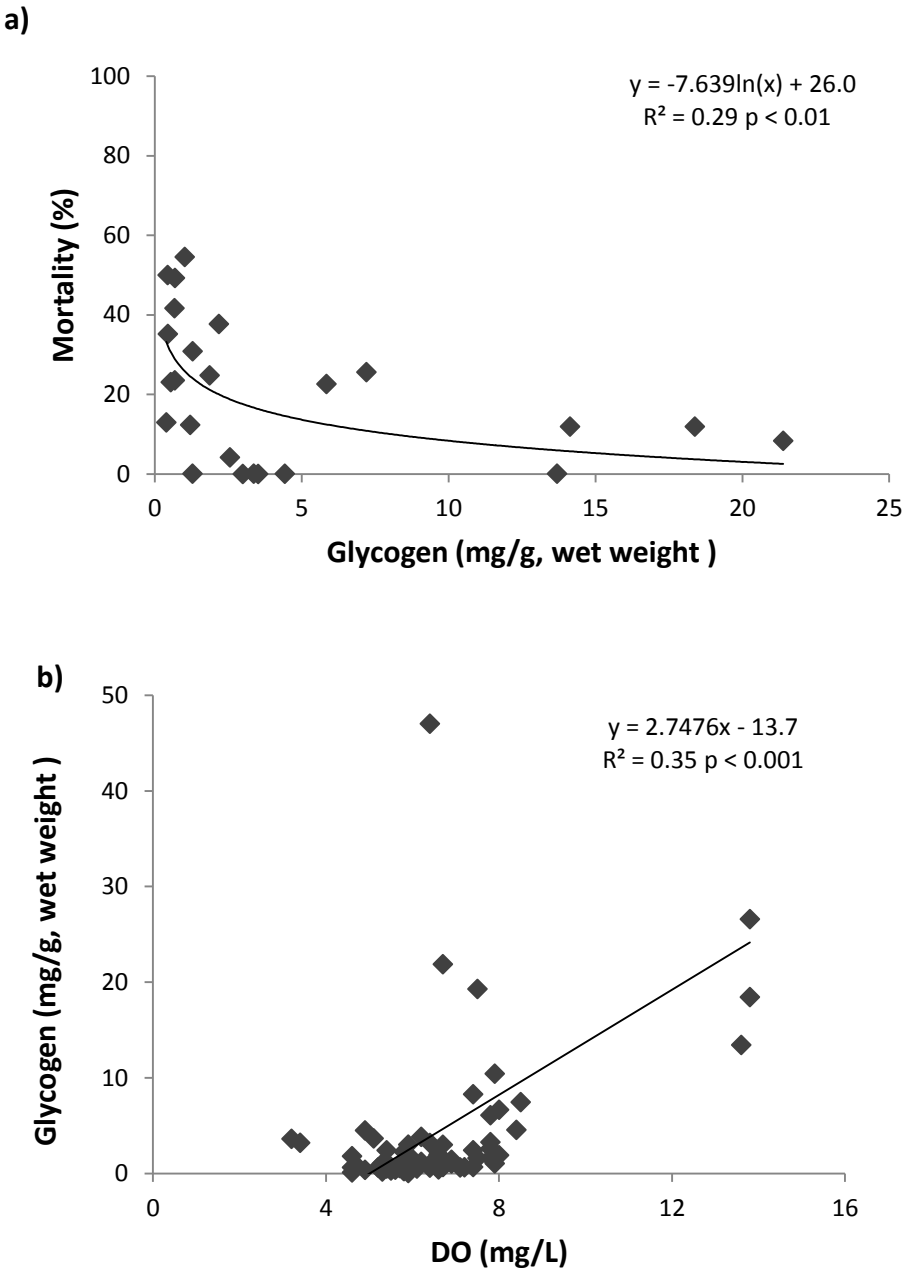


Figure 4-13: Relation between glycogen concentration (mg g^{-1}, wet weight) and: **a)** mortality (%); **b)** dissolved oxygen (mg L^{-1}).

Table 4-1: Nutrient concentrations at Sts. B, D and F in 2011 and 2012. Values under the detection limit are indicated by "<DL". "*" means samplings in which the aeration was off.

Sampling	Station	Depth (m)	$[NO_3^-]$ (µM)	$[NO_2^-]$ (µM)	$[PO_4^-]$ (µM)	$[SiO_2^-]$ (µM)
28 August 2011*	B	0	46.52	<DL	1.08	59.44
28 August 2011*	B	5	14.24	0.14	1.50	26.15
28 August 2011*	F	0	40.53	<DL	1.22	63.31
28 August 2011*	F	5	16.25	<DL	1.25	19.90
14 September 2011	B	0	14.57	0.14	1.27	24.84
14 September 2011	B	5	14.69	0.38	1.55	22.06
14 September 2011	F	0	14.81	<DL	1.15	24.14
14 September 2011	F	5	13.70	<DL	1.49	21.53
13 October 2011	B	0	15.69	<DL	1.27	32.69
13 October 2011	B	5	15.03	<DL	1.28	23.47
13 October 2011	F	0	16.20	<DL	1.23	44.81
13 October 2011	F	5	15.08	<DL	1.29	21.38
23 November 2011	B	0	0.79	<DL	0.23	19.49
23 November 2011	B	5	0.07	<DL	0.25	10.87
23 November 2011	F	0	1.23	<DL	0.20	32.67
23 November 2012	F	5	0.07	<DL	0.25	8.92
14 December 2011*	B	0	0.00	0.03	0.19	18.49
14 December 2011*	B	5	0.00	0.09	0.35	27.67
14 December 2011*	F	0	16.56	0.26	0.37	50.10
14 December 2011*	F	5	1.15	0.64	0.20	9.93
30 June 2012	B	0	20.11	0.32	0.02	58.92
30 June 2012	B	3	<DL	0.16	0.28	27.74
30 June 2012	B	5	1.15	0.26	0.48	32.62
30 June 2012	D	0	27.10	0.35	0.00	70.77
30 June 2012	D	3	<DL	0.16	0.18	24.05
30 June 2012	D	5	2.06	0.28	0.41	38.47
30 June 2012	F	0	48.25	0.39	0.02	95.78
30 June 2012	F	3	<DL	0.19	0.31	27.05
30 June 2012	F	5	1.60	0.27	0.40	31.51
31 July 2012	B	0	21.93	0.30	<DL	80.50
31 July 2012	B	3	<DL	0.03	<DL	4.55
31 July 2012	B	5	<DL	0.13	0.02	11.97
31 July 2012	D	0	5.88	0.17	<DL	86.64
31 July 2012	D	3	<DL	0.03	<DL	3.66
31 July 2012	D	5	<DL	0.06	0.16	9.52
31 July 2012	F	0	10.39	0.14	<DL	101.95
31 July 2012	F	3	<DL	0.02	<DL	3.51
31 July 2012	F	5	<DL	0.04	<DL	5.48

Table 4-1 continued: Nutrient concentrations at Sts. B, D and F in 2011 and 2012. Values under the detection limit are indicated by "<DL". "*" means samplings in which the aeration was off.

Sampling	Station	Depth (m)	$[NO_3^-]$ (µM)	$[NO_2^-]$ (µM)	$[PO_4^-]$ (µM)	$[SiO_2^-]$ (µM)
4 September 2012	B	0	0.40	0.08	0.16	5.86
4 September 2012	B	3	0.01	0.12	0.25	1.46
4 September 2012	B	5	0.10	0.35	0.41	3.44
4 September 2012	D	0	0.09	0.02	0.00	15.51
4 September 2012	D	3	<DL	0.05	0.18	0.00
4 September 2012	D	5	0.04	0.23	0.28	2.26
4 September 2012	F	0	<DL	0.02	0.08	16.78
4 September 2012	F	3	0.08	0.02	0.07	0.00
4 September 2012	F	5	0.15	0.26	0.65	5.46
2 October 2012	B	0	3.57	0.14	0.35	11.39
2 October 2012	B	3	0.81	0.08	0.31	2.80
2 October 2012	B	5	1.58	0.06	0.29	2.18
2 October 2012	D	0	21.60	0.24	0.65	84.14
2 October 2012	D	3	0.46	0.05	0.29	3.89
2 October 2012	D	5	0.75	0.10	0.36	4.14
2 October 2012	F	0	8.66	0.12	0.20	45.61
2 October 2012	F	3	0.589	0.09	0.25	2.58
2 October 2012	F	5	0.329	0.06	0.43	5.30
6 November 2012	B	0	12.62	0.29	0.78	30.48
6 November 2012	B	3	1.34	0.49	0.29	8.54
6 November 2012	B	5	1.08	0.45	0.22	7.56
6 November 2012	D	0	6.05	0.19	0.43	16.99
6 November 2012	D	3	1.55	0.36	0.24	8.54
6 November 2012	D	5	1.09	0.59	0.31	7.85
6 November 2012	F	0	16.29	0.21	0.81	38.79
6 November 2012	F	3	0.43	0.35	0.21	6.35
6 November 2012	F	5	1.56	0.58	0.31	9.29

Table 4-2: Mean oysters mantle C and N isotopes per station and depth in 2012 sampling. Traces indicate lost samples.

		July		September		October		November	
Station	**Depth (m)**	**$\delta^{15}N$**	**$\delta^{13}C$**	**$\delta^{15}N$**	**$\delta^{13}C$**	**$\delta^{15}N$**	**$\delta^{13}C$**	**$\delta^{15}N$**	**$\delta^{13}C$**
St A	0	8.98	-18.62	8.49	-17.45	9.49	-15.73	9.69	-17.54
	3	9.27	-18.80	8.56	-17.54	9.46	-17.33	-	-
	5	8.77	-17.80	8.45	-17.97	9.70	-15.70	10.78	-17.91
StB	0	8.75	-18.17	8.30	-17.76	8.99	-15.82	10.22	-17.80
	3	8.94	-19.31	8.37	-17.91	9.26	-16.29	10.21	-17.91
	5	9.27	-19.57	8.81	-18.07	9.76	-15.90	11.84	-17.86
StC	0	9.01	-18.76	7.84	-17.13	9.03	-15.92	12.23	-17.45
	3	8.94	-19.34	9.24	-18.28	9.20	-16.17	10.16	-17.34
	5	9.34	-18.61	9.87	-18.40	8.97	-16.17	10.10	-17.46
StD	0	9.27	-19.57	9.03	-17.46	9.31	-15.75	9.35	-17.62
	3	8.81	-20.02	9.53	-17.98	10.00	-16.19	10.26	-17.69
	5	8.98	-18.89	8.97	-17.22	9.80	-16.35	10.81	-17.59
StE	0	8.34	-17.89	9.24	-17.24	9.22	-15.87	9.85	-17.89
	3	8.75	-18.15	9.64	-18.28	9.37	-16.41	10.39	-18.24
	5	8.84	-16.78	9.83	-18.32	9.75	-16.19	10.00	-17.13
StF	0	8.64	-17.47	8.86	-17.12	9.62	-15.58	10.46	-18.03
	3	8.63	-19.02	9.19	-17.57	9.80	-15.91	10.82	-17.72
	5	9.20	-18.85	10.22	-18.13	9.90	-16.61	10.85	-17.88

Chapter 5.

Environmental relation with oyster production and possible applicability of artificial upwelling: a case study of a Brazilian aquaculture site

5.1 Introduction

To achieve the adequate growth bivalves primarily require a productive environment with adequate availability of phytoplankton and particulate organic matter (Silva, 1998), as well as a proper range of abiotic conditions, such as temperature and oxygen levels. However, the differences in culture performance are not only highlighted according to farming site environmental characteristics but also by the applied management. Suitable crop management determine the farm economical feasibility. Combined knowledge of the effects of culture area environmental characteristics on oysters and farming methods will eventually lead to insights of the most useful management techniques.

Nowadays, new techniques as artificial upwelling, have been tested as promising tools for management of shellfishes activities and positive results on food concentration, temperature and dissolved oxygen have been proved (as shown in Chapters 3 and 4). Temperature and food availability are believed to be important

factors for oyster production, thus it is of extreme importance to determine exactly in which ways these environmental parameters affect the oyster production in its several stages. Because abiotic conditions fluctuate along culture period and may reach harmful levels for oysters, this specific knowledge may assist application of the correct mitigative technology.

5.2 Objectives

The present survey investigated possible relationships between oyster performance and variation in temperature and food availability in order to further obtain knowledge to support mitigation plans for common farming problems. For this, it was used as a case study available production data of a farm located in the main Brazilian bivalve farming region: South Bay, Santa Catarina Island and State. Possible applications of artificial upwelling related to oyster performance and management technique were also discussed.

5.3 Materials and methods

5.3.1 Study site

Oyster grow-out in suspended vertical devices (lantern nets) installed at the

island side of the South Bay (Ribeirão da Ilha), Santa Catarina State, Brazil (27°44' S; 48°33' W), was considered for the survey (Fig. 5-1). The study site is located in the Southern portion of the channel (7 km width, 1 m to 9 m depth) comprised between the continental border and the Santa Catarina Island, characterized by calm waters with some small river discharges and minimum tide variation (Cruz, 1998; Knopper, 2002). The bay may be reached by upwelled cold nutrient rich waters (16-17°C and salinity 35.5 ‰) from South Atlantic Central Water (SACW) in periods of favourable northeastern winds. The region is also influenced by the La Plata River (34°54' S; 57°00' W) plume that may spread northward to Santa Catarina State coast (Piola et al., 2000). In the South Bay, hypoxia is not a problem, but summer high temperature and low food availability are limiting factors for oyster good performance.

5.3.2 Farm management

Oyster farming cycle started with seeds obtained from a public laboratory to be grown in the sea in the traditional 'long-line' system. The long-line employed consisted of a cable which floats on the surface, supported by plastic buoys. Hanging from these cables towards the bottom are the oyster lanterns, typical culture equipment for the grow-out phase in Brazil. Oyster production numbers at commercial level from different crop years (2005, 2006, 2007 and 2008) were obtained from Atlântico Sul Marine Farm (www.fazendamarinha.com.br). The farm comprises ca. 35 ha of suspended parallel long-lines with 100 meters length each and 10 meters distance between them. Grow-out at the sea consisted of 4 different classes according to

individual oyster shell length: seed (> 3 mm), juvenile (> 3 cm), adult (> 5cm) and marketable (7-12 cm). Marketable size class is further divided to satisfy market needs in small, medium, large and extra large oysters. Unlike Japanese aquaculture, in Brazil oysters are all separately as individuals throughout the grow-out phase and different culture devices may be employed according to oyster size class (Fig. 5-2). Oyster seeds from same genetic origin were purchased from a local public hatchery (Laboratory of Marine Molluscs, Federal University of Santa Catarina, Florianópolis State) at sizes 3 and 5 mm mean length. Crops were yearly initiated with seed stocking in floating nursery trays between March and April, and successive monthly stockings may be done throughout a year. Each seed stocking comprises an allotment, and several allotments in a year will constitute the crop. For the present survey only one allotment of each yearly crop (March or April seed stocking) was considered and thus the terms ‘allotment’ and ‘crop’ may be used as synonyms.

Farm management routine included growing devices taken out from the sea in intervals of 15 to 30 days with individuals separated by different sieve sets and sorting strategies. Individuals grown to the next size class were reallocated according to the rearing sequence (Fig. 5-2) and those which have not grown sufficiently to pass to the next step were returned to the same equipment back in the sea. After reaching the last size class (marketable) oysters were visually sorted according to size in a selection table. In the assessed region, a crop regularly extends oyster outputs up to 1.5 year since initial seed stocking. The present study focused on oyster performance at juvenile, adult and marketable size classes.

Four annual crops were assessed: 2005/06 stocked in April, and 2006/07, 2007/08 and 2008/09, stocked in March. Three and five millimetres seeds were stocked for the crops initiated in 2005, 2006 and 2008, and 5 mm seeds for the crop started in 2007. Data from these specific crops were selected due to standardized farm management protocol, and same seed genetic origin. Thus, differences among annual outputs were assumed be mostly related to environmental effects. The asynchrony in oyster development under sea farming conditions generates several outputs of a certain size class in the course of the crop period. Oyster productive performance was determined during crops according to different rearing phases: seed to juvenile (phase 1), juvenile to adult (phase 2) and adult to marketable (phase 3). Relative outputs of each size class were computed and expressed as survival (%) in relation to the initial oysters stocked in the phase. Final crop survival was calculated as the ratio between total marketable oyster output and initially stocked seed number**.**

5.3.3 Environmental data

Data on water temperature and chlorophyll *a* concentration in the farming area were compiled for time series concerning oyster crops in order to check for possible relationship with farming performance. Daily sea surface temperature (SST) values were determined in a single local station (27°44’ S; 48°33’ W) within the farming area using a manual thermometer at 50 cm depth. Remote sensing temperature data in the farming area were obtained from ANTARES Project was corrected by *in situ*

determined temperature. The availability of remote sensing data was obviously dependent on absence of clouds coverage and this was the main reason why some days in crop time series had no available data. Chlorophyll *a* concentration was assessed by remote sensing outputs of ocean colour (ANTARES Project) and also corrected by in situ data.

5.3.4 Statistical analysis

Pearson correlation tested the significance of relationships found in regressions, as denoted by determination coefficient (R^2) and p value. It was applied for remote sensing *versus in situ* regressions, as well as for relationships involving survival, phase interval, temperature and chlorophyll *a* values.

5.4 Results

5.4.1 Environmental data

Seasonal features in SST were clearly showed in the crop time series. Maximum mean values of SST were obtained in March (25.9 ± 1.1 °C) whereas the lowest value varied between July (19.5 ± 1.8 °C) and August (19.5 ± 1.4 °C) (Silveira

Jr. *et al.*, 2008a). Colder months showed higher SST variation among winters (Fig. 5-3a). The year of 2007 showed the highest monthly mean temperature in March (27.1 °C) and also lowest monthly mean temperature in July (16.2 °C). Mean and standard deviations of SST was 21.8 ± 3.3; 22.3 ± 3.7, 22.1 ± 4.4, and 22.1 ± 2.8 °C for the crops 2005/06, 2006/07, 2007/08 and 2008/09, respectively.

On the other hand, available chlorophyll *a* levels showed no apparent annual pattern in the present time series with values ranging from 0.4 (April 2007) to 11.8 µg L^{-1} (May 2008) (Fig. 5-3b). From the available data set, the occurrence of elevated chlorophyll *a* values (> 5 µg L^{-1}) was overall higher in 2007 followed by 2008, and to lesser extent 2006 and 2005. Mean and standard deviations of chlorophyll *a* levels was 2.75 ± 2.0, 3.14 ± 2.5, 3.97 ± 2.9 and 3.9 ± 3.0 µg L^{-1} for the crops 2005/06, 2006/07, 2007/08 and 2008/09, respectively.

5.4.2 Environmental effects upon oyster performance

Seeds were stoked in relatively elevated seawater temperatures and chlorophyll *a* levels (Fig. 5-3a, b), except for crop of 2005/06 where seed stocking temperature was < 22 °C. High SST during *C. gigas* seed stocking and in the adjacent rearing months resulted in reduced survival in the seed-juvenile rearing phase, as observed in crops 2006/007, 2007/08 and to a lesser extent in 2005/06 (Table 1, Fig. 5-3a). The highest survival at seed-juvenile phase (66.9 %) was verified in the 2008/09 crop. Chlorophyll *a* levels at seed stocking were apparently not related to survival at

seed-juvenile phase, since decreased survival in 2007/08 occurred even at relatively high chlorophyll *a* concentration (Fig. 5-3b). However, elevated chlorophyll *a* levels may have also contributed to outstanding increased survival in juvenile-adult phase in this crop.

Temperature showed to be a crucial factor for survival in early stages of *C. gigas* farmed in this sub-tropical farming area. Though not significantly, mean temperature in the rearing phase showed a trend to be negatively related to crop survival especially in the stages seed-juvenile and juvenile-adult, with temperature values ranging between 20.0 and 21.3 ºC, and 19.6 and 20.9 ºC, respectively (Fig. 5-4a).The survival in the adult-marketable grow-out phase was not affected by temperature at 22.0 - 23.1 ºC interval. The development rate of farmed oyster, as assessed by phase interval (months) in different rearing phases, showed not related to mean phase temperature under these ranges (data not shown). Although the temperature seems to be within the optimum condition specified in Chapter 2, it is possible that the degree of variations of temperature in short time period along culture is also a factor to take into account in the survival of oysters. On the other hand, chlorophyll *a* influenced the development rate of *C. gigas* under the assessed farming conditions with significant effects upon the time spent in each rearing phase (Fig. 5-4b). The negative relationship observed between phase duration and mean chlorophyll *a* concentration was highly significant in the assessed crops for seed-juvenile and adult-marketable phases, at ranges of 2.37 and 3.33, and 2.87 and 4.26 µg L^{-1} mean chlorophyll *a* levels, respectively. Though not significant, oyster development and mean chlorophyll *a* also showed a trend of negative correlation in

the juvenile-adult phase, under ranges of 2.14 and 3.35 μg L^{-1}. Oyster survival showed no relationship with mean phase chlorophyll *a* concentration at determined intervals (data not shown).

5.4.3 Relationships between final survival and phase survival

Final crop survival showed significantly affected by the performance in the seed-juvenile phase. When final crop survival was correlated with phase survival, the significant correlation only showed for the phase 1 ($R^2 = 0.96$, $p = 0.02$, Table 5-1).

5.5 Discussion

5.5.1 Mortality and environmental factors

Grow-out of farmed bivalves in the ocean is highly dependent on environmental characteristics as well as exposed to environmental changes, which highlights a certain degree of vulnerability. In the present 2005-2009 field survey four single allotments (first allotments of the crop) from each yearly commercial crop were chosen due to its unique characteristics: (1) management followed a similar protocol therefore differences between crops management were minimized; (2) oysters were

taken from the sea as soon as marketable size was achieved to supply the market, thus were not stocked back in the sea for future trade which could elevate mortality; (3) oysters were obtained from the same hatchery and lineage thus genetic interference is believed to be minimized.

Elevated temperatures have been suggested as a negative factor in oyster survival causing, especially in summer, the so-called summer mass mortalities (Goulletquer et al., 1998; Malhan et al., 2009). On the other hand, it may be attributed to multi-factorial effects, such as the combination of low water quality and elevated temperatures (Dégremont et al., 2007; Malhan et al., 2009; Soletchnik et al., 2005; Wolff, 2007). In addition, high temperatures are also likely to facilitate pathogen infections in oyster early life stages (Burge et al.,2007; Gagnaire et al., 2006). *C. gigas* seeded during relatively elevated temperatures may thus be underperformed in comparison to those stocked in relatively lower temperatures periods, emphasizing the importance of careful selection of seeding time (Burge et al., 2007).

In the present survey when the seeding was done in March (month of higher recorded temperatures) the survival was not as good as the survival obtained from the seeding in April (2005/06 crop). Oyster in phases 2 and 3 may be not as affected by temperature possibly because successive selection of more temperature resistant individuals from the end of phase 1 to the next phases. This may have resulted in non-significant difference in phase 3 survivals among crops, and survival of adult-marketable individuals showed not affected by the determined environmental factors. Chlorophyll *a* may have improved survival by either mitigating the high temperature effects on bivalves or enhancing survival when temperature was already

at adequate range. The component however has revealed of secondary importance to final oyster survival.

5.5.2 Growth and environmental factors

Growth rates will evidently affect profit in bivalve aquaculture. Food availability is the main responsible factor for bivalve growth (Ren and Ross, 2001; Gosling, 2004; Ren et al., 2010) despite some evidence for no correlation between them has also been reported (Gangnery et al., 2003). A large variety of primary producers is accessible to bivalve´s diets such as benthic and pelagic microalgae. Though *C. gigas* is reported to be an opportunistic filter-feeder, phytoplankton is recognized as the major contributor to the diet (Leal et al., 2008). In the present study, high chlorophyll *a* concentrations had the expected positive effect upon oyster development in all culture phases, so that development periods could be related to oyster growth. The data indicated no significant effect of temperature upon oyster growth, as also verified in previous studies (Spencer, 2002; Gangnery et al., 2003).

5.5.3 Implications for the artificial upwelling application

Decreased temperature and food availability were also results on the environment induced by an artificial aeration as described in Chapters 3 and 4 of this

thesis. Among the results, it was found that one of the most fruitful management for oyster survival appears to be that in which seeding is performed in colder temperature. Duration of cultured phase and fast achievement of commercial size however, depend on the available food during the cultured period. In spite of that, in order to attend the demand of the market commercial farms might choose less suitable periods for seeding, a divergence from the logical approach. Therefore, new technologies should be developed taking into consideration the reality of commercial culture. Skilfully designed artificial upwelling could guarantee better water quality at the grow-out period by lowering the water column temperatures.

At the study site, maximum mean temperatures reached 25.9 ± 1.1 °C, lower than Omura Bay values. However, in case Brazilian aquaculture significantly spread northwards to warmer coastal locations mean temperature values may reach values higher than 30 °C. Artificial upwelling was also shown to increase quality and quantity of phytoplankton. Because chlorophyll *a* concentration was related to the duration of culture phase, aeration could induce a faster performance of oysters.

Moreover, due to the clear importance of the seed-juvenile phase for the final crop performance, the artificial aeration if performed during initial rearing phases could contribute significantly for a higher aquaculture production.

5.6 Conclusions

Important environmental features impact oyster farming in Southern Brazil, two main effects were assessed: seawater temperature and chlorophyll *a*. The former showed to affect mainly survival, negatively related to yield. Chlorophyll *a* acted as a secondary factor influencing growth, positively related to it and showed a high relation with phase interval. Artificial upwelling might be a good strategy to shape the final production characteristics since it has proved to change both temperature and chlorophyll *a* concentrations positively in relation to farming requirements, especially if it is performed in the decisive early culture stage. These results are extremely important because to mitigate and find adaptive strategies for problems concerning oyster management production it is necessary previous knowledge of the area. This knowledge, afterwards, and together with studies on management tools and designs, could enable effective decision-making by responsible agencies and sectors.

Figure 5-1: Oyster farming location (circled), Ribeirão da Ilha, Santa Catarina Island, Santa Catarina State, Brazil.

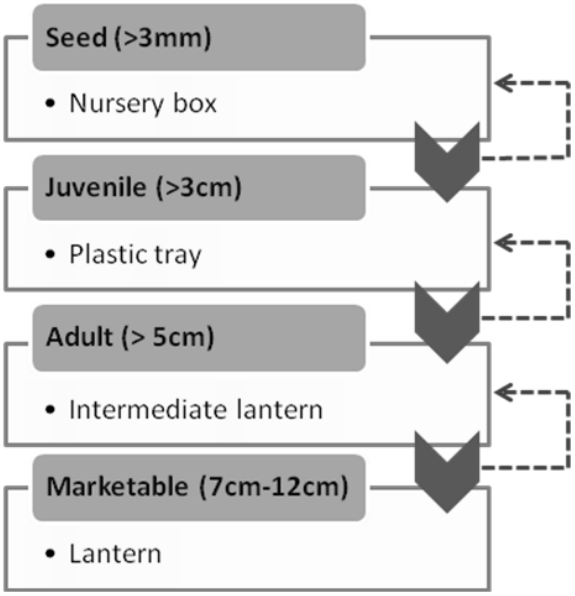


Figure 5-2: Explicative scheme of the different oyster culture steps and the in which the oysters are cultured in each step, according to the oyster shell size. Dotted arrows show the possibility of oysters return to the same device if under-grown to the next step.

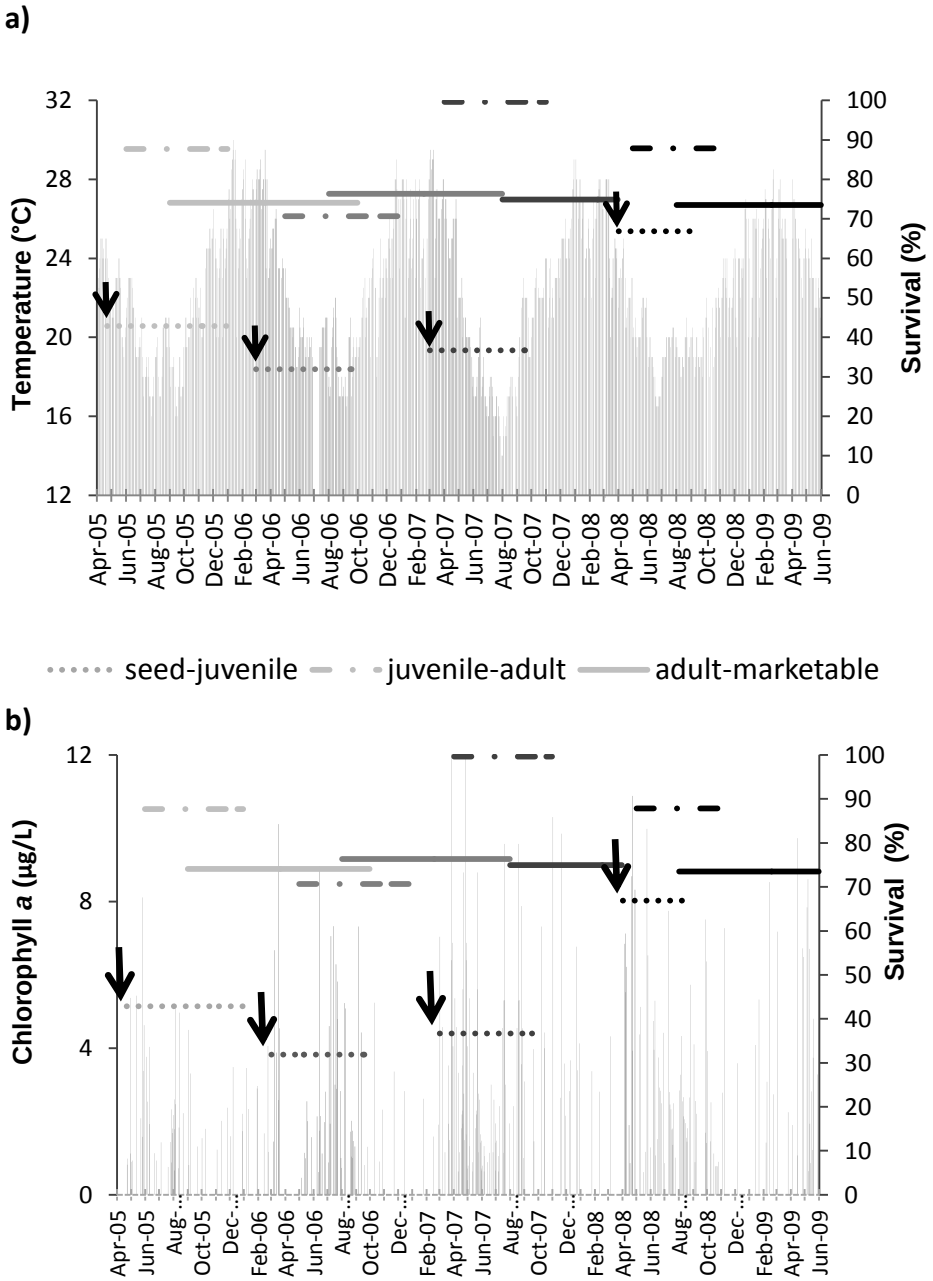


Figure 5-3: Temperature **a)** and chlorophyll *a* **b)** variation in the four oyster (*Crassostrea gigas*) crops (2005/06, 2006/07, 2007/08 and 2008/09) and comparison with survival in the different culture phases in South Bay, Santa Catarina Island, Brazil. Horizontal lines: total survival in relation to individual stocked and time interval of each culture phase. Black arrows indicate the time of seed stocking for each crop.

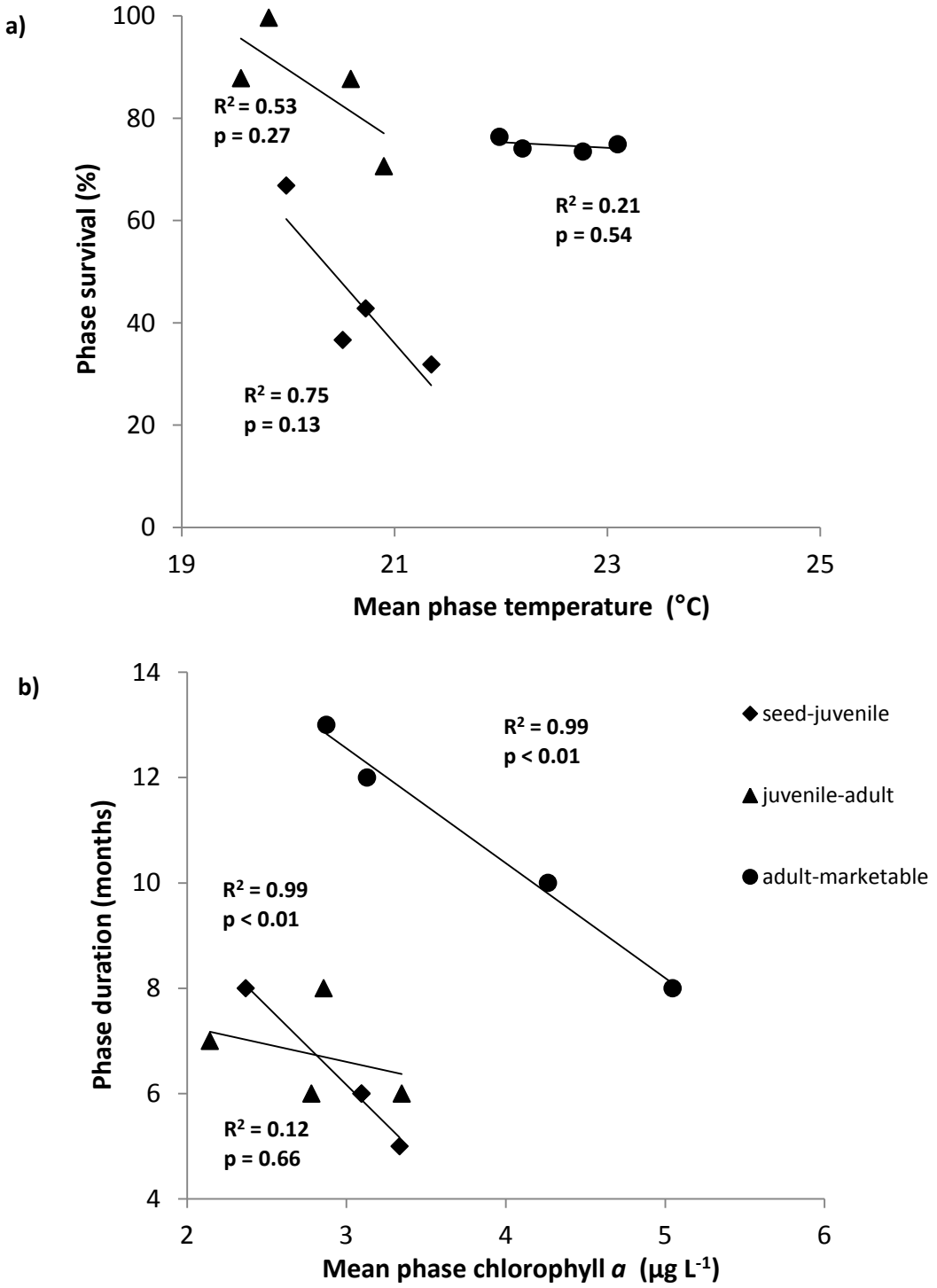


Figure 5-4: The relationship between: **a)** survival and temperature, and **b)** phase duration and chlorophyll *a*, in different culture phases of oyster (*Crassostrea gigas*) farmed in South Bay, Santa Catarina Island, Brazil.

Table 5-1: Values of R^2 and P from correlations (n = 4) between final survival and phase survivals. Significant correlations are underlined ($p \leq 0.05$).

	R^2	p
final survival x survival seed-juvenile phase	0.96	<u>0.02</u>
final survival x survival juvenile-adult phase	0.17	0.58
final survival x survival adult-marketable phase	0.18	0.57

Chapter 6.

General Discussion

6.1 Novelty of the study on new alternatives for shellfish farm environment improvement

Many works have studied the shell movements of bivalves related to environmental stressors (e.g. Tran, 2010). However, there is a lack of knowledge of how synergetic effects of environmental parameters can affect oyster behaviour. In this work, the first approach was to study possible combined effects of temperature and oxygen levels, which could cause oyster stress and make them susceptible to mortality, like the ones occurring in Omura Bay every summer. The results proved the necessity of mitigating technologies to avoid oyster production loss. In this work, the chosen studied mitigating technology was artificial upwelling.

Recent works have focused in analyzing the implications of artificial upwelling on nutrient concentration and phytoplanktonic biomass for possible application for aquaculture areas. These studies, however, have not investigated the direct impact of the upwelling on the filter feeders, because they were not performed in the aquaculture area. I attempted to analyze in a deeper level, not only the effects of the circulation induced by the upwelling, but also how could it directly affect, if the case, oyster growth,

survival and health. To our knowledge, this is the first attempt to check oyster performance under artificial upwelling aeration systems. Therefore the work contributes to the real possible applicability of the aeration system instead of only speculating the results it would have in cultures as previous works did.

Finally, I also attempted to show possible applicability of artificial upwelling for different commercial farms which are operated by different culture managements. During the whole sampling phase, meetings were held not only with fisherman who owned the farms, but also involving the company with which the aeration design was developed and the prefectural research institute which supported the study development. This is an extremely rare approach in scientific studies, because unfortunately there is a critical lack of communication between academia, stakeholders and farmers. Thus, the work was developed seeking not only scientific importance but also commercial relevance.

6.2 Synthesis of major findings and conclusions of the study

In this work, both the stressful summer conditions and a way to overcome those were investigated. Although the oxygen concentration was expected to be the main factor for oyster mortalities in Omura Bay, it was found that oxygen played a secondary role to the effects of temperature both in laboratory experiments and field observations in the study sites. However, extremes of temperature and low oxygen had negative synergetic effects on oysters. Investigations on oyster shell behaviour were not

compartmented, but were assembled the periods of changing conditions, that were shown to be critical for understanding the oyster behaviour. Shell movement analysis confirmed that under natural conditions in Omura Bay oysters experience both temperature and oxygen extremes that do not favour their development. Unfortunately, most environmental ranges include bad conditions in many faming locations around the world, where summer mortality is a constant farming threat. Water quality was improved by performing an artificial upwelling in local, as clearly shown by decreased temperature, food quality and quantity improvement, oyster CI and others. Care is needed however, when linking these effects directly to oyster development, because the final oyster production is affected if the water quality deteriorates at any farming stage. This was shown in the summer peak when our aeration could not overcome the high temperature and increase oxygenation concentration. Aquaculture farms should consider artificial upwelling techniques especially at early development stages, since it is known to be the most sensitive and defining stage of the production.

6.3 Future challenges

This work has several limitations and technical problems that serve as challenges for future researches. Troubles with aeration cables, loss of equipments and the sampling frequency are results of natural conditions, consisting of difficult problems to be solved in a limited period of time. In future works, one of the main challenges is to

find an ideal aeration design and flow rate which can support the best results found in this research along the farming-period.

The aeration performed in this study failed to overcome the peak of summer high temperature and hypoxic formation, especially in the middle of Omura Bay. New attempts need to be made in other target areas with different aeration designs, including combination of aeration in sites located near freshwater discharges, because it is possible for these factors to increase oyster performance.

The sampling frequency of 30-days interval did not allow the detailing of mortalities period, thus the exactly period when positive aeration effects were overcomed by the summer negative effects were unknown. This might be important information for managing aeration rate within the changes in the season and should be among the aims of future investigations.

New laboratory experiments of oyster shell movement research should be designed to examine the deeper synergetic effects of temperature and oxygen on spiking and closure of shells. Other possible environmental distressors of the farming area should also be analyzed, to allow the future use in commercial farms. The real-time knowledge of stressful condition would allow the determination of the performing period of the artificial upwelling.

Lastly, aeration could also be investigated taking an approach towards the problem of global warming in forthcoming studies. Oschlies et al (2010) pointed that because nutrient-rich upwelled water would fertilize surface ocean layers and stimulate carbon sequestration by photosynthetic product and a portion may sink out and be

removed from contact to atmosphere. By lowering the temperature of sea surface waters, artificial upwelling would also lead to cooling of overlying air and terrestrial primary production and to a higher extent, and heterotrofic respiration would be decreased and carbon sequestered. Although those authors' results are very hypothetical, it shows an additional importance of the study of artificial upwellings.

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